Iegūts apmaiņā pret
Acta Horti Botanici.

1241

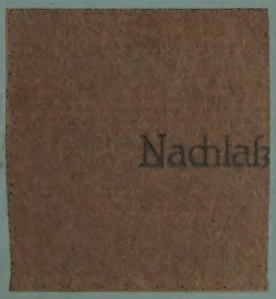
ACTA HORTI BERGIANI. BAND 11. N:O 7.

REDUCTION DIVISION, POLLEN LETHALITY AND POLYPLOIDY IN APPLES

BY

OTTO HEILBORN

WITH 10 FIGURES IN THE TEXT



UPPSALA 1935
ALMQVIST & WIKSELLS BOKTRYCKERI-A.-B.
35437

1241.

7. Reduction Division, Pollen Lethality and Polyploidy in Apples.

By

OTTO HEILBORN.

Nachlaß von Prof. N. Malta

A. Introduction.

Modern interest in the cytology of apples arose from inquiries into sterility and incompatibility. Originally, the investigations into sterility served a purely practical purpose. The results were laid down in a voluminous literature, which has added a good deal to our knowledge of pollen germination and self-sterility, and has directed our attention to the problems of reproduction in fruit-trees, without, however, arriving at any solution of these problems. This literature has been compiled and reviewed by several authors, e. g. FLORIN (1927), ZIEGLER and BRANSCHEIDT (1927), KOBEL (1931) and ELSSMANN and v. VEH (1931). A critical treatment has been presented by the present writer (HEILBORN 1932).

The introduction of cytology into pomological investigations involved — as in many other fields of biologic research — a renewal of problems and points of view. The papers by RYBIN (1926 and 1927) were the first to establish definitely the existence of two kinds of apple varieties, diploids and triploids, the former with relatively good, the latter with bad pollen. Additional evidence, though less accurate, was furnished by SHOEMAKER (1926) and KOBEL (1926 and 1927). Further data on chromosome numbers were published by CRANE and LAWRENCE (1929), as well as by the present writer (HEILBORN 1928 and 1930), the last-mentioned author also showing the reduction division in the pollen mother-cells to be sensitive to high temperatures. Further important details are found in some papers by NEBEL (1929 *a*, *b* and *c*).

Cytologic research into apples and pears entered upon a fresh phase by the publication of the paper by DARLINGTON and MOFFETT (1930) on »secondary polyploidy» and secondary chromosome balance. This paper introduced the study of secondary chromosome association and multivalents in apples. Apples and pears and, later on, all Pomoideae (MOFFETT 1931), are now regarded as polyploids. This view appears to be generally accepted, though many details, especially as to the secondary association of chromosomes, are disputed or

doubted (NEBEL 1930, 1933, SAX 1931, 1932, 1933, ADATI 1933). Still further data on chromosomes in apples and pears have lately been furnished by NATIVIDADE (1932), ROSCOE (1933), MIEDZYRZECKI (1933, 1934) and MOFFETT (1934).

Studies on the embryology of triploid apples have been published by ELSSMANN and v. VEH (1931), HOWLETT (1932), STEINEGGER (1932), v. VEH (1933 *a* and *b*) und GORCZYŃSKI (1934). Important embryological and cytological data on *Sorbus* have recently been added by LILJEFORS (1934).

The chromosome studies in apples have proved the existence of two kinds of phenomena causing pollen lethality, viz. triploidy and sensitiveness to high temperatures, the latter being of minor importance. These two agencies are, however, by no means sufficient to explain all the sterility actually found. The writer, based on a statistical study of the occurrence of empty pollengrains i various apple varieties, therefore suggested a further explanation, that of gametic lethals (HEILBORN 1932). This interpretation has turned out to fit the theory of »secondary polyploidy» remarkably well. The same holds good for the extensive investigations into incompatibility in apples carried out for many years by CRANE (CRANE and LAWRENCE 1929, 1930 *a* and *b*, 1931). The theory of incompatibility in polyploids has been further developed by LAWRENCE (1930), and the irregular behaviour of incompatibility in apples is in good consonance with this theory. Finally, the results of genetical analysis, recently published (CRANE and LAWRENCE 1933), tend to prove the existence in apples of numerous polymeric factors. As polymery is mostly connected with, and caused by, polyploidy, these genetic researches also agree with the theory of »secondary polyploidy».

As is seen, the various investigations into apples, reviewed above, have begun with the phenomena of pollen sterility and incompatibility, proceeded to cytology, and, finally, terminated in the fundamental problem of polyploidy. The irregularity and somewhat enigmatic character of many of the phenomena of reproduction and heredity in apples appear to be well explained by the polyploid nature of these fruit-trees.

The writer's own investigations into apples which have been carried on since 1926 have followed the same course as outlined above. The results have partly been published (conf. above). The first paper (1928) dealt with chromosome conjugation und triploidy and the bearing of these phenomena on pollen sterility; the second paper (1930), on the influence of high temperatures upon chromosome conjugation, was somewhat beside the principal line of investigation; the third paper (1932) dealt with pollen lethality as an outcome of polymeric lethals. Some additional data on pollen lethality are, finally, to be found in a recent popular report (HEILBORN 1933 *b*).

During all these years the writer has continued to collect and investigate fresh material, trying to make the elucidation of the problems more and more complete. As far as possible I have also tried to form opinions of my own as regards the doubtful questions. Through the simultaneous efforts of so many investigators the problems of meiosis, sterility, incompatibility and heredity in apples — as looked upon from the point of view of polyploidy — now appear

to have ripened, thus enabling a comprehensive treatment of the topic. The present work is an attempt at such a treatment, where many unpublished data of my own are presented and the problems discussed in connection with the results of other workers.

The writer's material has mostly been collected in Hortus Bergianus at Stockholm, some material for cytologic research being kindly furnished by the gardens at Alnarp. I wish to tender my sincere thanks to Prof. ROB. E. FRIES of Hortus Bergianus and to Mr EMIL JOHANSSON of Alnarp. A couple of fixations have been made in private gardens at Djursholm, near Stockholm. The cytologic material has been fixed in NAWASHIN's fluid and stained in iron haematoxylon.

B. Reduction Division.

1. Chromosome Numbers.

Through the cytologic investigations, reviewed above, the chromosome numbers are now known of numerous apple varieties. All these varieties fall into two groups, diploids with $2n = 34$ and triploids with $3n = 51$ chromosomes. Data on supposed aneuploidy, published especially in earlier papers, have turned out to be erroneous, and aneuploid apple varieties most certainly do not exist. The »Kola» is reported as tetraploid with $4n = 68$ (NEBEL 1930), but this variety is an (artificially produced) hybrid between a cultivated apple and the wild American crab apple, and does not belong to the cultivated varieties in a proper sense. The conclusion, therefore, seems safe that all cultivated apple varieties are either diploid or triploid.

In table I I have collected data on chromosome numbers now available except those given in the paper by NATIVIDADE (1932) which, unfortunately, I have not seen. The table contains 148 diploids and 33 triploids. For comparison the reader is referred to the tables given by KOBEL (1931, p. 115) on »gute Pollenspender» and »schlechte Pollenspender», a classification based on the germination percentages of the pollen. Almost half of the varieties in KOBEL's tables have now been investigated cytologically. As is seen, the classification according to chromosome numbers shows very close correspondence to that based on pollen germination. The good pollen producers are diploids and the poor ones are triploids. According to both classifications $^4/_5$th—$^5/_6$th of all apple varieties are diploids and $^1/_5$th—$^1/_6$th triploids.

Table I. **Diploid and Triploid Apple Varieties.**

A. *Diploid Varieties (2n = 34).*

Variety	*Author*	*Variety*	*Author*
Aderslebener Calville	Nebel	Cellini	Heilborn Kobel
Åkerö	Heilborn	Charlamovsky (Duchess)	Heilborn Nebel Roscoe
Alexander	Heilborn	Cox's Orange Pippin	Kobel Crane & Lawrence Darl. & Moffett
Allington Pippin	Crane & Lawrence Darl. & Moffett Heilborn	Cox's Pomona	Darl. & Moffett Heilborn
Annie Elizabeth	Crane & Lawrence	Danziger Kantapfel	Kobel
Antonovka	Rybin	Dash-Alma	Rybin
Antonovka-Kamenitchka	Rybin	Deacon Jones	Roscoe
Apfel aus Lunow	Nebel	Delicious	Nebel Roscoe (Shoemaker)
Aport	Rybin	Der Boehmer	Nebel
Astrachan, big transp.	Heilborn	Djir Hadzhi	Rybin
» , red	Nebel	Dolgo	Nebel
» , white	Heilborn Kobel Rybin	Duchess Favourite	Darl. & Moffett
Babushkino	Rybin	Early Red Bird	Roscoe
Barlóvskoje	Rybin	Early Victoria	Crane & Lawrence Darl. & Moffett
Baumanns Reinette	Moffett (Kobel)	Eden	Nebel
Beauty of Bath	Crane & Lawrence Darl. & Moffett	Eneroth's Klaräpple	Heilborn
Belle Fille	Miedzyrzecki	Esopus Spitzenberg	Kobel
Belleflower, Yellow	Rybin Nebel	Fenouillet jaune	Miedzyrzecki
Belleflower × Kitaika	Rybin	» rouge	Miedzyrzecki
Belle Joséphine	Miedzyrzecki	Géante des Expositions	Miedzyrzecki
Belyi Naliv	Rybin	Geheimerat Dr Oldenburg	Nebel
Ben Davis	Nebel	General von Hammerstein	Nebel
Berner Rosenapfel	Kobel	Golden Reinette of Kursk	Rybin
Blanche d'Espagne (Kalmar glasäpple)	Miedzyrzecki	Golden Russet	Roscoe
Borowinka-Borowitsky	Miedzyrzecki	Grenadier	Crane & Lawrence
Calville blanc	Miedzyrzecki	Grimes Golden	Nebel Roscoe
» du Roi	Rybin	Grågylling	Heilborn
» Grand Duke of Baden	Nebel	Gule Penbe	Rybin
» Lesans	Nebel Miedzyrzecki	Halland's Brunnsäpple	Heilborn
» rouge	Miedzyrzecki	Hampus	Heilborn
Candille Sinap	Rybin		
Carlisle Pippin	Crane & Lawrence		

Table I (cont.).

Variety	*Author*	*Variety*	*Author*
Irish Peach	Crane & Lawrence Darl. & Moffett	Red Siberian Crab	Nebel
Jonathan	Nebel Roscoe	Reine des Reinettes	Miedzyrzecki
Kasseler Reinette	Kobel	Reinette de Champagne	Rybin
Kavlås	Heilborn	Reinette d'Oberdieck	Rybin
Kentish Codlin	Crane & Lawrence	Reinette d'Orléans	Rybin
Keswick Codlin	Crane & Lawrence Darl. & Moffett	Reinette pain de Sucre	Miedzyrzecki
Lane's Prince Albert	Nebel Crane & Lawrence Darl. & Moffett	Reinette rouge d'Hiver	Roscoe
Lord Derby	Crane & Lawrence	Reinette très tardive	Miedzyrzecki
Lord Grosvenor	Rybin	Reinette Zuccamaglio	Crane & Lawrence
Macoun	Nebel	Rev. W. Wilks	Moffett
Maglemer	Heilborn	Rival	Crane & Lawrence
Mank's Codlin	Crane & Lawrence Nebel	Rome	Nebel
Margaretaäpple, red	Heilborn	Rosenhäger	Heilborn
Mc Intosh	Nebel	Rosmarin blanc	Rybin
Medina	Nebel	Roter Jungfernapfel	Nebel
Melba	Nebel	Roter Stettiner	Rybin
Melon	Heilborn	Sary-Sinap	Rybin
Milton	Nebel	Sary-tursh-Alma	Rybin
Minister von Hammerstein	Nebel	Sävstaholm	Heilborn
Muskat-Reinette	Kobel	Signe Tillisch	Heilborn
Newfane	Nebel	Skvoznoy Naliv	Rybin
Newton Wonder	Crane & Lawrence	Sommerrambour	Nebel
Northern Spy	Crane & Lawrence Darl. & Moffett	Sommergewürzapfel	Kobel
Öland's Kungsäpple	Heilborn	Spätblühender Taffetapfel	Nebel
Ontario-Reinette	Kobel Nebel	Stäringe	Heilborn
Oranie	Heilborn	Stenkyrka	Heilborn
Oslins	Crane & Lawrence	Suislepper	Rybin
Patte d'Oie	Miedzyrzecki	Svanetorp	Heilborn
Pearmain d'Adam	Miedzyrzecki	Tchernoguz	Rybin
Pfirsichroter Sommerapfel	Kobel	Tchulanovka	Rybin
P. J. Bergius	Heilborn	Titovka	Rybin
Rambour de Himbsel	Miedzyrzecki	Transparente blanche	Heilborn
» of Tsarkoje Selo	Rybin	Transparente de Croncel	Kobel Heilborn
Red Spy	Roscoe	Twenty Ounce	Nebel
		Wagner	Roscoe
		Vaughan Seedless	Nebel
		Wealthy	Heilborn Nebel
		Weidner's Goldreinette	Nebel
		Wellington	Nebel Roscoe

Table I (cont.).

Variety	*Author*	*Variety*	*Author*
Winesap	Nebel	Worcester Pearmain	Crane & Lawrence Darl. & Moffett
Winter Banana	Roscoe		
Winter Golden Pearmain	Rybin	Yellow Newtown	Nebel
		Yellow Richard	Heilborn Nebel
Winter Gray Reinette	Rybin		
Winter Majetin	Crane & Lawrence	Yellow Transparent	Nebel
Vitgylling	Heilborn	York Imperial	Roscoe
Wolf River	Roscoe	Zelenka, Crimean	Rybin
148 diploid varieties cytologically investigated.			

B. *Triploid Varieties (3n = 51).*

(those marked with an asterisk have not been very precisely counted but their triploidy is regarded as being beyond doubt).

Variety	*Author*	*Variety*	*Author*
Arkansas	Nebel	Jaques Lebel	Kobel
Baldwin	Darl. & Moffett Nebel Kobel	Menznauer Jägerapfel*	Kobel
		110 Nomblot	Miedzyrzecki
Blenheim Orange	Crane & Lawrence Darl. & Moffett Kobel Nebel	Nonpareil	Roscoe
		Reinette grise de Vitry	Miedzyrzecki
		Reseda-Reinette*	Kobel
		Rhode Island Greening	Nebel
Bohnapfel*	Kobel	Ribston Pippin	Crane & Lawrence Nebel Heilborn Kobel
Bramley's Seedling	Crane & Lawrence Darl. & Moffett		
Canadian Reinette	Rybin (Kobel)	Rossvik*	Heilborn
Crimson Bramley	Darl. & Moffett	Roter Eiserapfel*	Kobel
Damason-Reinette*	Kobel	Schöner von Boskoop	Nebel Kobel
Fallawater	Roscoe		
Frösåker	Heilborn	Stäfner Rosenapfel*	Kobel
Genet Moyle	Crane & Lawrence	Stark	Roscoe
Gravensteiner (several varieties)	Nebel Kobel Heilborn Roscoe	Stayman Winesap*	Shoemaker
		Tompkin's King	Nebel
		Warner's King*	Kobel
Harbart's Reinette*	Kobel	Washington	Moffett
Hurlbut*	Heilborn	Winter-Zitronenapfel*	Kobel
Husmodersäpple*	Heilborn		
33 triploid varieties cytologically investigated. (»Gravensteiner» comprises several varieties!).			

2. Chromosome Conjugation in Diploids.

There has been some diversity of opinion regarding chromosome conjugation in diploid apples. According to data given by RYBIN (1927) 17 gemini of a normal appearance are seen at diakinesis, as well as at first metaphase in pollen mother-cells, though at diakinesis the partners of some of the gemini are figured as lying close together rather than actually conjugated. The present writer (HEILBORN 1928, 1930) found the formation of gemini at first division to be normal, except in those cases where the flower buds had been exposed to high temperatures. Such temperatures caused a failure — total or partial — of gemini formation and the appearance of univalents. In pollen mother-cells of »White Astrachan» a few univalents were observed already at such a low temperature as + 20° *C.*, in pollen mother-cells of »Åkerö» at + 25° *C.*, of »Cox's Pomona» at + 26° *C.* These temperatures are by no means excessive and suggest the regular occurrence of a few univalents at normal open-air temperature. In a similar way, MIEDZYRZECKI (1933, 1934) reports the appearance of univalents in his material as due to exceptionally high spring temperatures during 1933. ROSCOE (1933) reports regular behaviour at meiosis, except a »slight lagging» in a few cases. KOBEL's papers do not give any clear information as to gemini formation in diploids, and the papers by NEBEL and ADATI seem to show their material to be regular.

In their important paper DARLINGTON and MOFFETT (1930) describe the regular and rather abundant occurrence of quadrivalents and sexivalents at metaphase I of diploids, and they also illustrate a few diakinetic stages. Their descriptions and illustrations clearly suggest the existence of true multivalents. Lagging univalents were likewise reported as occasionally occurring. In a later paper, however, MOFFETT (1931) more precisely discriminates between true (primary) conjugation and secondary association or secondary pairing, and states that true multivalents »do occur, although not with such frequency as would appear to be indicated from the evidence of the polar views» (l. c. p. 429). »Further evidence that the chromosome grouping observed at metaphase is of a »secondary» nature is obtained from observations of diakinesis. In the great majority of divisions examined 17 bivalents were observed, though in a small percentage multivalent associations were found» (l. c. p. 431). In a quite recent paper, MOFFETT (1934) still reduces the importance of true multivalents. They are said to be wholly absent in pears. ROSCOE (1933), though with some hesitation, mentions a few doubtful cases of quadrivalents. SAX (1932) and ADATI (1933), on the other hand, dispute the findings of MOFFETT and maintain that true multivalents (due to chiasma formation) most probably are lacking.

The interest taken in these multivalents pertains to the problem of polyploidy, especially the question of auto- or allopolyploidy.

According to the writer's experience metaphases of apples are not well suited for the study of multivalents on account of the strong secondary association, which makes the distinction between »primarily» and »secondarily» associated chromosome groups very difficult. I have, therefore, confined the search

for multivalents to diakinesis. The results are found in table II, which also contains data on univalents at both diakinesis, metaphase I and anaphase I. All the material covered by the table has been fixed at low or moderate temperature.

The »grand totals» in the last column of the table do not supply a correct idea of the real frequency of multivalents, as in these totals metaphases and anaphases are included as well (and which have not been analyzed with regard to multivalents). The table shows that out of 95 diakineses only 6 show multivalency, and that these 6 nuclei have only one quadrivalent each. More complex multivalents have not been found, nor trivalents. Thus 6 % of the diakinetic nuclei show quadrivalency — but less than 0.4 % of the gemini have united to quadrivalents. Hence quadrivalents appear to be a regular, though very

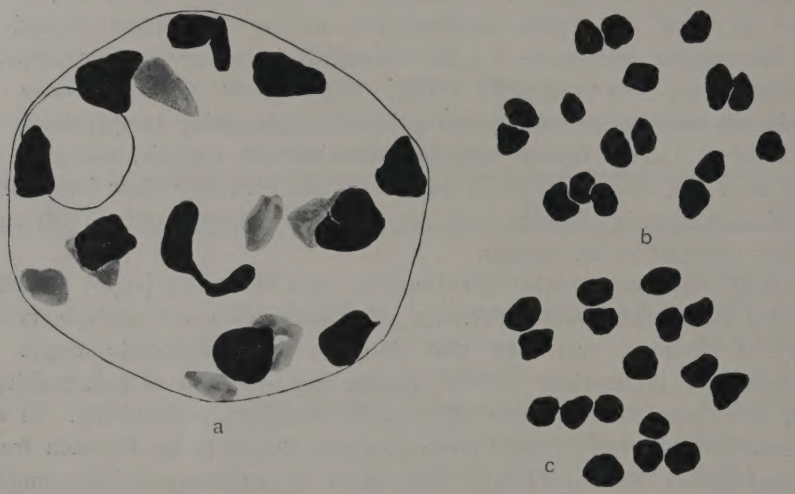


Fig. 1. a Cellini, diakinesis (17_{II}). — b Cellini, metaphase I ($1.3_{II}+5.2_{II}+4.1_{II}$). — c Cellini, metaphase I ($2.3_{II}+4.2_{II}+3.1_{II}$). — 5000×.

rare, occurrence. The shape of a few of these quadrivalents is shown in fig. 2. b, f and g.

At diakinesis univalents are by no means of such rare occurrence. At metaphase I, on the other hand, they are practically lacking at normal open-air temperature — not a single case is found in the table — but they reappear as »lagging» chromosomes at anaphase I. At diakinesis the univalents often lie in pairs, in more or less close proximity but without chiasma-formation (conf. above concerning RYBIN), in other nuclei, again, they may lie far apart (fig. 2. b and k). The former type of univalents might have been occasioned by a too strong terminalization, resulting in the final resolution of the »terminal chiasmata», or both types of univalents may owe their existence to a total failure of chiasma-formation. In the latter case, the force that has kept the univalents together in pairs, might, perhaps, be the same as that calling forth secondary association at metaphase (conf. below).

Table II. **Chromosome Conjugation in Diploids.**

Variety	*Stage*	*Conjugation*					*Number of nuclei*	*Number of gemini*
		17_{II}	$1_{IV}+15_{II}$	$1_{IV}+14_{II}+2_{I}$	$16_{II}+2_{I}$	$15_{II}+4_{I}$		
Cellini	Diakinesis	21	2	—	7	1		
	Metaphase I	15	—	—	—	—		
	Anaphase I	11	—	—	2	—		
Number of nuclei	Total	47	2	—	9	1	59	1003
White Astrachan	Diakinesis	10	1	—	3	1		
	Metaphase I	16	—	—	—	—		
	Anaphase I	6	—	—	1	—		
Number of nuclei	Total	32	1	—	4	1	38	646
Rosenhäger	Diakinesis	12	—	1	4	1		
Number of nuclei	Total	12	—	1	4	1	18	306
Åkerö	Diakinesis	2	—	—	—	—		
	Metaphase I	12	—	—	—	—		
	Anaphase I	—	—	—	1	—		
Number of nuclei	Total	14	—	—	1	—	15	255
Öland's Kungs-äpple	Diakinesis	11	1	—	—	—		
Number of nuclei	Total	11	1	—	—	—	12	204
Eneroth's Klar-äpple	Diakinesis	5	—	—	2	—		
Number of nuclei	Total	5	—	—	2	—	7	119
Stäringe	Diakinesis	2	1	—	—	—		
	Metaphase I	4	—	—	—	—		
Number of nuclei	Total	6	1	—	—	—	7	119
Other varieties	Diakinesis	7	—	—	—	—		
	Metaphase I	14	—	—	—	—		
	Anaphase I	1	—	—	—	—		
Number of nuclei	Total	22	—	—	—	—	22	374
Grand total		149	5	1	20	3	178	3026

Recently, LINDSTROM and HUMPHREY (1933) have called attention to exactly the same phenomenon in tomatoes. Here, too, paired univalents occur at diakinesis but disappear at metaphase I. The phenomenon was rather rare in pollen mother-cells of diploid plants, but quite common in (artificially produced)

tetraploids. It would, therefore, seem as if these univalents, both in tomatoes and apples, were in some way or other connected with the polyploidy of the plants. Though the chiasmata in these cases obviously have been completely resolved, this does not interfere with the regularity of the metaphase plates. Metaphase pairing, therefore, does not appear to depend solely upon chiasmata (as necessitated by DARLINGTON's theories). Anaphase disjunction, on the other hand, apparently is disturbed by the lack of chiasmata.

Table II shows that out of 178 nuclei investigated 24 have 2—4 univalents. Hence, 13 % of the nuclei contain univalents, but less than 1 % of the gemini are resolved into univalents. — In this connection reference is made to HOLLINGSHEAD's (1932) work on *Triticum vulgare*.

Finally, a few remarks should be added regarding the nucleolus. Besides the 17 gemini a small, spherical body is very often — but not always — found in close connection with the nucleolus (fig. 2. b, f and h), in a few cases such a body, rarely two, are seen lying separated from the last-mentioned. The bodies stain with haematoxylon. The writer has no idea as to their nature. Perhaps they might have something to do with satellites, but we are not well enough acquainted with the morphology of apple chromosomes to pronounce any opinion on this question. In a few pollen mother-cells satellite-like structures have really been observed (fig 2. i). Besides, almost always one geminus, or a few of them, lie closely connected with the nucleolus, as has been reported regarding satellite-bearing chromosomes in other plants.

3. Secondary Chromosome Association in Diploids.

DARLINGTON and MOFFETT (1930) discovered the secondary association of the chromosomes in apples. According to these authors the maximum association — regarded as the most significant one — is, 10, which corresponds to the formation of 7 groups of bivalents at metaphase I. This observation is the basis of their interpretation of the Pomoideae as trebly hexasomic tetraploids (conf. below), with the original basic number 7.

This conclusion has been doubted by several authors. Thus, NEBEL (1930) considers that though »post-diakinetic clumping» might be an indication of homology it may, instead, just as well be »mechanical in origin». The author's suggestions (NEBEL 1930, 1933) as to the mechanism producing the »clumping» seem, however, somewhat speculative and difficult to test. It seems also difficult to understand how 7 »main tracts» (in the plasma) for chromosome movement could allow such a great variability of grouping as is really found. On the other hand, URSULA HELM's (1934) recent, rather remarkable results regarding »gesetzmässige Lage der Gemini im Kernraum» tend to show that the grouping of the chromosomes in the nuclei of a diploid is managed by some very accurate mechanism of an unknown nature. However, the mechanism is obviously such as to give, in a tetraploid, a similar grouping, but now with homologous chromosomes in close proximity to one another. This would result in secondary

association of homologous chromosomes, and such a secondary association must be taken as a proof of polyploidy.

Furthermore, secondary associations are said to be often seen in »distinctly diploid and monosomic» species, but the species cited by NEBEL as example, *Mentha Requienii,* might be open to doubt, as the fundamental set of 9 in the

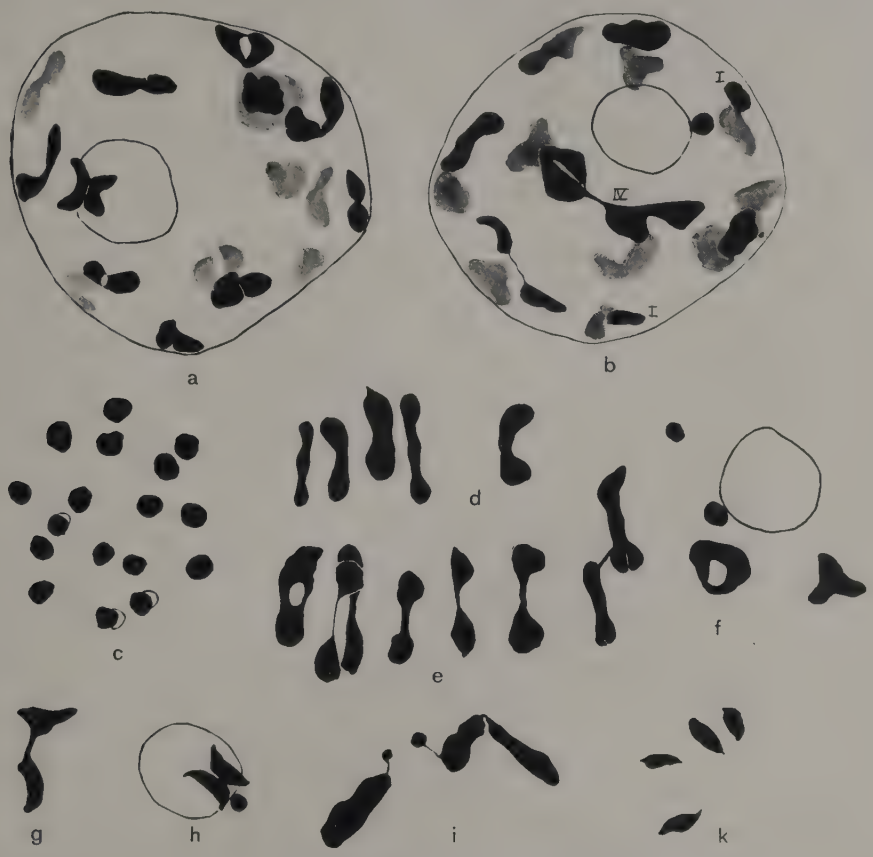


Fig. 2. a Big transparent Astrachan, diak. (17_{II}). — b Rosenhäger, diak. ($1_{IV}+14_{II}+2_{I}$+a small body at nucleolus). — c Åkerö, metaph. I ($1.3_{II}+5.2_{II}+4.1_{II}$). — d Åkerö, metaphase I, gemini. — e Alexander, metaph. I, gemini. — f White Astrachan, diak. (detail): $1_{IV}+2_{II}+2$ small bodies and nucleolus. — g Stäringe, diak., quadrivalent. — h Rosenhäger, nucleolus with one geminus and small body. — i Rosenhäger, 2 gemini with satellite-like structures (diak.). — k Rosenhäger, diak., 2 pairs of univalents. — 5000×.

genus *Mentha* may well be, itself, a secondarily balanced derivative of a still more primitive set.

SAX (1931) declares that »this clumping of chromosomes, especially in sectioned material, may have little significance», but he does not offer any reason for his verdict. On the whole, criticisers, curiously enough, appear to prefer the word »clumping» which entails an erroneous conception of the phenomenon. In

well-fixed cells there is, in fact, no clumping at all, only a rather widely spaced grouping of the gemini (conf. fig. 1—2; conf. also LAWRENCE 1931, p. 370).

NEBEL (l. c.) also raises the objection that DARLINGTON and MOFFETT analyzed and figured cultivated varieties only. Later, however, MOFFETT (1931) presented similar data on several wild species of Pomoideae. The fact that secondary associations appear to be less evident in some species than in others, of apparently identical constitution — it is, for instance, less pronounced in pears than in apples (MOFFETT 1934) — does probably not interfere with the general importance of the phenomenon. In this connection AFIFY's (1933 *a*) recent findings regarding the hybrid *Lycopersicum esculentum* × *racemigerum* should be quoted. This author found a rather strong secondary association into groups of two in the hybrid, while such association was lacking in both parents.

NEBEL's further objection that in the work by DARLINGTON and MOFFETT »the phenomenon of perfect triple hexasomy and quadruple tetrasomy was exhibited in only 5 per cent of the cases» deserves special mention. The curves given in the present paper, as well as the curves on secondary association in *Brassica*, recently presented by CATCHESIDE (1934), prove the existence of a variation more or less closely following the law of chance. If the maximum association in apples results in the formation of 7 groups of gemini, the minimum association in 17 groups, the mean value will then be 12 groups. If an association into more than 12 groups occurs just as often as an association into less than that number, the frequency curve will be symmetrical, and the upper limit of the curve — representing the maximum association — will show a value not exceeding a few per cent of the total number of observations. In cultivated apples this value is now 3 %, in *Brassica napus* less than 1 %. This is a quite natural consequence and does not present any difficulty to the theory. Plants apparently exist with a much weaker association, in extreme cases giving a unilateral curve with the mode at the minimum value (conf. GUSTAFSSON 1934, table IV). In the reverse case, plants with exceedingly strong association and the mode at the point of maximum association might also be thought of as a possibility, though their existence seems more than questionable.

The critical comments by ADATI (1933) are more in the nature of personal belief than scientific argumentation. No statistics are presented. On the other hand, the careful treatment of the problem by MÜNTZING (1933) on potatoes and MEURMAN (1933) on *Acer* has put the connection between secondary association and polyploidy beyond question (conf. below p. 171).

The objection has, finally, been raised against DARLINGTON's and MOFFETT's conclusions that their statistics are too scanty to be relied upon. In order to remove this deficiency, to some extent at least, I myself have tried to procure some further data. As it is necessary to avoid misinterpretations, I have selected for counting only such plates of metaphase I as do not show any contraction of the plasma. Such a contraction is easily induced by the fixative and makes the limits of the chromosome groups indistinct. The difficulty of finding cells without plasma shrinkage has made my statistics rather meagre but, on

Table III. **Secondary Chromosome Association in Diploids.**
(polar views of metaphase I)

Variety	*Number of groups*	*Formula*	*Meta-phases counted*	*Number of groups*	*Formula*	*Meta-phases counted*
Cellini	8	$4 \cdot 3_{II} + 1 \cdot 2_{II} + 3 \cdot 1_{II}$	1	9	$1 \cdot 3_{II} + 6 \cdot 2_{II} + 2 \cdot 1_{II}$	2
	8	$2 \cdot 3_{II} + 5 \cdot 2_{II} + 1 \cdot 1_{II}$	2	10	$1 \cdot 3_{II} + 5 \cdot 2_{II} + 4 \cdot 1_{II}$	2
	9	$3 \cdot 3_{II} + 2 \cdot 2_{II} + 4 \cdot 1_{II}$	3	12	$1 \cdot 3_{II} + 3 \cdot 2_{II} + 8 \cdot 1_{II}$	1
	9	$2 \cdot 3_{II} + 4 \cdot 2_{II} + 3 \cdot 1_{II}$	3	12	$5 \cdot 2_{II} + 7 \cdot 1_{II}$	1
White Astrachan	8	$3 \cdot 3_{II} + 3 \cdot 2_{II} + 2 \cdot 1_{II}$	1	10	$1 \cdot 3_{II} + 5 \cdot 2_{II} + 4 \cdot 1_{II}$	2
	8	$2 \cdot 3_{II} + 5 \cdot 2_{II} + 1 \cdot 1_{II}$	1	10	$7 \cdot 2_{II} + 3 \cdot 1_{II}$	1
	9	$2 \cdot 3_{II} + 4 \cdot 2_{II} + 3 \cdot 1_{II}$	2	11	$6 \cdot 2_{II} + 5 \cdot 1_{II}$	3
	9	$1 \cdot 3_{II} + 6 \cdot 2_{II} + 2 \cdot 1_{II}$	1	12	$5 \cdot 2_{II} + 7 \cdot 1_{II}$	1
Åkerö	9	$2 \cdot 3_{II} + 4 \cdot 2_{II} + 3 \cdot 1_{II}$	1	10	$1 \cdot 3_{II} + 5 \cdot 2_{II} + 4 \cdot 1_{II}$	1
	9	$1 \cdot 3_{II} + 6 \cdot 2_{II} + 2 \cdot 1_{II}$	1	11	$1 \cdot 3_{II} + 4 \cdot 2_{II} + 6 \cdot 1_{II}$	1
	10	$3 \cdot 3_{II} + 1 \cdot 2_{II} + 6 \cdot 1_{II}$	1	11	$6 \cdot 2_{II} + 5 \cdot 1_{II}$	1
	10	$2 \cdot 3_{II} + 3 \cdot 2_{II} + 5 \cdot 1_{II}$	1	12	$5 \cdot 2_{II} + 7 \cdot 1_{II}$	1
Other varieties	7 (?)	$3 \cdot 3_{II} + 4 \cdot 2_{II}$	1	10	$7 \cdot 2_{II} + 3 \cdot 1_{II}$	1
	8	$2 \cdot 3_{II} + 5 \cdot 2_{II} + 1 \cdot 1_{II}$	2	11	$1 \cdot 3_{II} + 4 \cdot 2_{II} + 6 \cdot 1_{II}$	1
	9	$2 \cdot 3_{II} + 4 \cdot 2_{II} + 3 \cdot 1_{II}$	2	11	$6 \cdot 2_{II} + 5 \cdot 1_{II}$	1

Number of groups	7	8	9	10	11	12	
» » metaph.	1	7	15	9	7	4	Total 43

Number of gemini in a group	1	2	3
Number of groups	157	194	62
Number of groups (calculated)	—	172	129

the other hand, these statistics do not differ materially from those of DARLINGTON and MOFFETT. Table III and the graphs of fig. 3 give the data.

I have not found the maximum association into 7 groups more than once in 43 plates, and even this case seemed somewhat doubtful. It might have been interpreted as an association into 8 groups. The last column of the table contains a comparison with values theoretically calculated on the basis of DARLINGTON's and MOFFETT's theory ($3 \times 3_{II} + 4 \times 2_{II}$). The data observed differ from those calculated by showing fewer groups of 3, more groups of 2 and a considerable number of single gemini. This depends of course upon the variable strength of the associations and does not present anything surprising. More complex associations than groups of 3 have not been found.

Graph a of fig. 3 contains the data of table I of the paper by DARLINGTON and MOFFETT (1930). The data are, however, given in a reverse order as compared with the table. The table gives the numbers of secondary associations

of bivalents — as do also the tables and graphs of CATCHESIDE, l. c. — the graph, again, gives the numbers of chromosome groups. The maximum number of 10 associations corresponds to the minimum number of 7 groups. Plotted

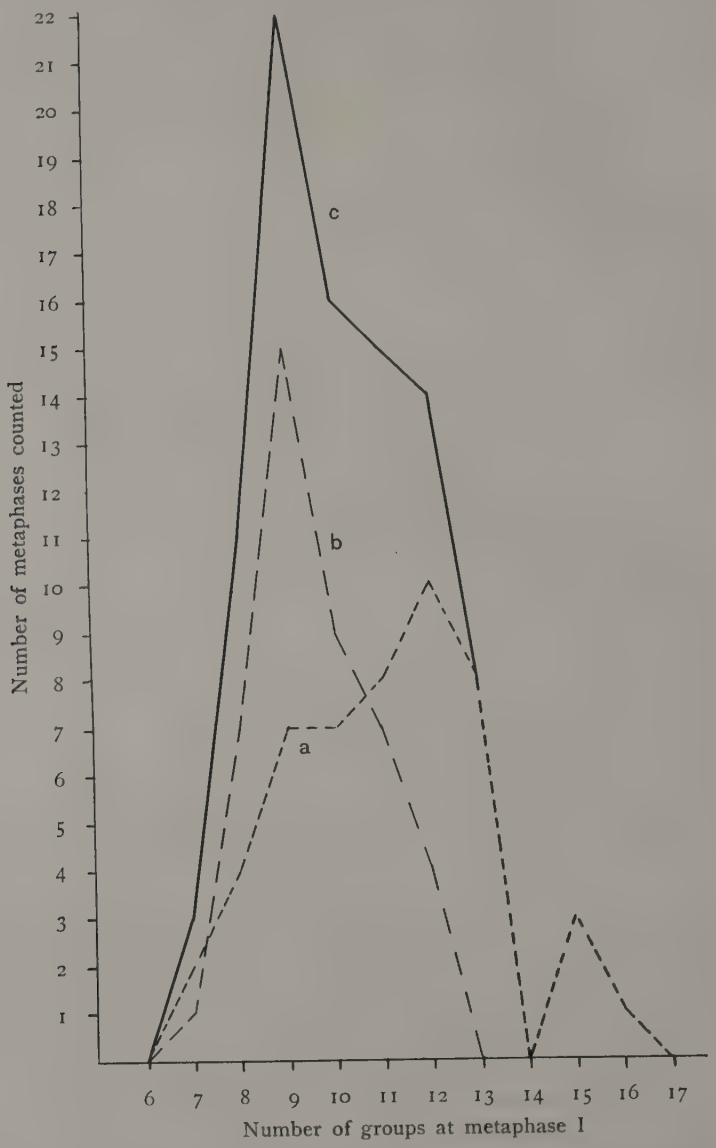


Fig. 3. Secondary chromosome association: number of groups at metaph. I. Graph a: DARLINGTON and MOFFETT. — b author. — c Both combined.

in this way, the graph supplies more direct information as to the various chromosome arrangements presented in the microscope, as well as to the supposed primary basic number 7. Graph b gives in the same way the data of table III of the present work; and in graph c all data are brought together.

There is a difference between DARLINGTON's and MOFFETT's results and my own regarding the mode of the curves, but it seems uncertain whether this difference has any real significance. The general shape of all three curves tends to confirm the conclusion that the maximum association is 10, corresponding to the formation of 7 groups of gemini.

According to LAWRENCE (1931, p. 362) »maintenance of pairing through diakinesis is conditioned solely by the maintenance of chiasmata between pairs, i. e. only those chromosome pairs which are materially connected will survive the repulsion phase of diakinesis». The existence of univalents in pairs at diakinesis of apples and tomatoes (conf. above) appears to necessitate some revision of the preceding statement. To the same class of observations possibly belong those by CATCHESIDE (1932) on a haploid *Oenothera*. In this plant chromosome conjugation at metaphase I appears to be 2 ½ times as frequent as at diakinesis. This indicates a metaphase pairing not depending on chiasmata. A somewhat similar phenomenon is the secondary association between univalents reported in some recent publications. The first clear case of this phenomenon seems to have been that of the triploid *Pyrus minima*, presented by MOFFETT (1931, p. 444).

Recently, GUSTAFSSON (1934, 1935) published data on secondary association between homologous univalents in apomictic *Taraxacum*-forms, and speaks of »pseudogemini» as resulting from such association (in contradistinction to »eugemini» with chiasma-formation). This definition of ideas might be accepted provisionally, but GUSTAFSSON's theoretical interpretation of the nature and causes of secondary association is entirely speculative, and his suggestion that secondary association is »due to terminal affinity and to the pellicles around the chromosomes being fused» (1934) lacks support of critically analyzed observations.

The fine threads connecting some of the univalents of *Taraxacum* — upon which much stress is laid — probably do not differ from similar threads found in other material. I have myself seen such connecting strands between bivalents in pollen mother-cells of *Carex*. In some fixations (apparently somewhat inferior) they are plainly stainable and visible, in others they are not discernable to the eye but may appear on a photographic plate. In zoological literature such fine strands have often been figured, as, for instance, in the classical drawings of the chromosome groups of *Anasa*, *Protenor* and *Lygaeus* (conf. WILSON 1925, chapt. X). In all these cases they may connect the chromosomes in every direction and have nothing to do with »terminal affinity» nor — probably at least — with chromosome pellicles. They may, perhaps, result from the coagulation of the colloidal ground substance of the nucleus, under the influence of the fixative.

GUSTAFSSON's statement regarding his interpretation of the phenomenon (1934): »That this is really the case I consider established by this enquiry», merely discloses a lack of judgement. On the whole, the last-mentioned paper is replete with »statements» without proof, and purely hypothetical forces (such as »terminal affinity») or observations of uncertain significance (such as chromosome pellicles) are dealt with as if they were clear and generally accepted

scientific facts. In this connection reference is once more made to the paper by URSULA HELM (conf. above). There is little reason for further discussing the mechanics of secondary association in the present paper. If possible, the writer will revert to this subject in a later work.

4. Meiosis in Triploids.

RYBIN (1927) was the first to describe the meiosis of a triploid apple. This work, however, was inconclusive as regards chromosome conjugation, since only anaphase I and metaphase II were studied, but not diakinesis and metaphase I. With the exception of some incomplete data, published by KOBEL (1927) and the writer (HEILBORN 1928), the first data on chromosome conjugation in triploid apples were those furnished by NEBEL (1929) and DARLINGTON and MOFFETT (1930).

NEBEL reported all gradations of pairing from 17 trivalents to 25 bivalents plus one univalent. This last-mentioned extreme was interpreted as resulting from allosyndetic pairing between two sets of 17 and autosyndesis within the third set. In view of later investigations the type $25_{II} + 1_{I}$ would now seem somewhat doubtful. Trivalents are probably always found in triploids.

DARLINGTON and MOFFETT presented new data (l. c., table II). Trivalents, bivalents and univalents were found in every nucleus investigated, the maximum value of trivalent formation being $15_{III} + 2_{II} + 2_{I}$. In most of the nuclei 1—3 quadrivalents were seen; one nucleus also contained 1 sexivalent plus 1 septivalent; another nucleus 1 sexivalent plus 1 nonovalent. The total number of chromosomal units in metaphase I plates varied from 15 to 24, most of the plates showing 19—23 units. The occurrence and frequency of sexi-, septi- and nonovalents may appear open to some doubt. Associations higher than trivalents show that autosyndesis within a group of seventeen occurs to a certain extent. Univalents often lag at anaphase I and divide.

In his work 1931 MOFFETT distinguishes between two types of triploids in Pomoideae, according to their behaviour at meiosis: (1) Forms in which the pairing is almost complete, rarely more than three or four univalents being found lagging at anaphase (as in *Pyrus malus*). (2) Forms in which pairing is not so regular, as many as 14 univalents (sometimes still more) being observed. Trivalents are in these cases relatively rare, though not altogether lacking. The gemini arrange themselves in the centre of the plates at metaphase I, surrounded by the univalents in a ring round the periphery. Secondary associations occur between the gemini in a manner comparable to that described in diploids. The difference between the two types of triploids clearly is only that between autotriploids and allotriploids. That the triploid apples are (secondary) autotriploids, is shown not only by their behaviour at meiosis but also by RYBIN's finding of triploid plants in the offspring of diploid varieties (RYBIN 1927). The triploid apple varieties of our gardens have probably originated in this way, and are thus autotriploids.

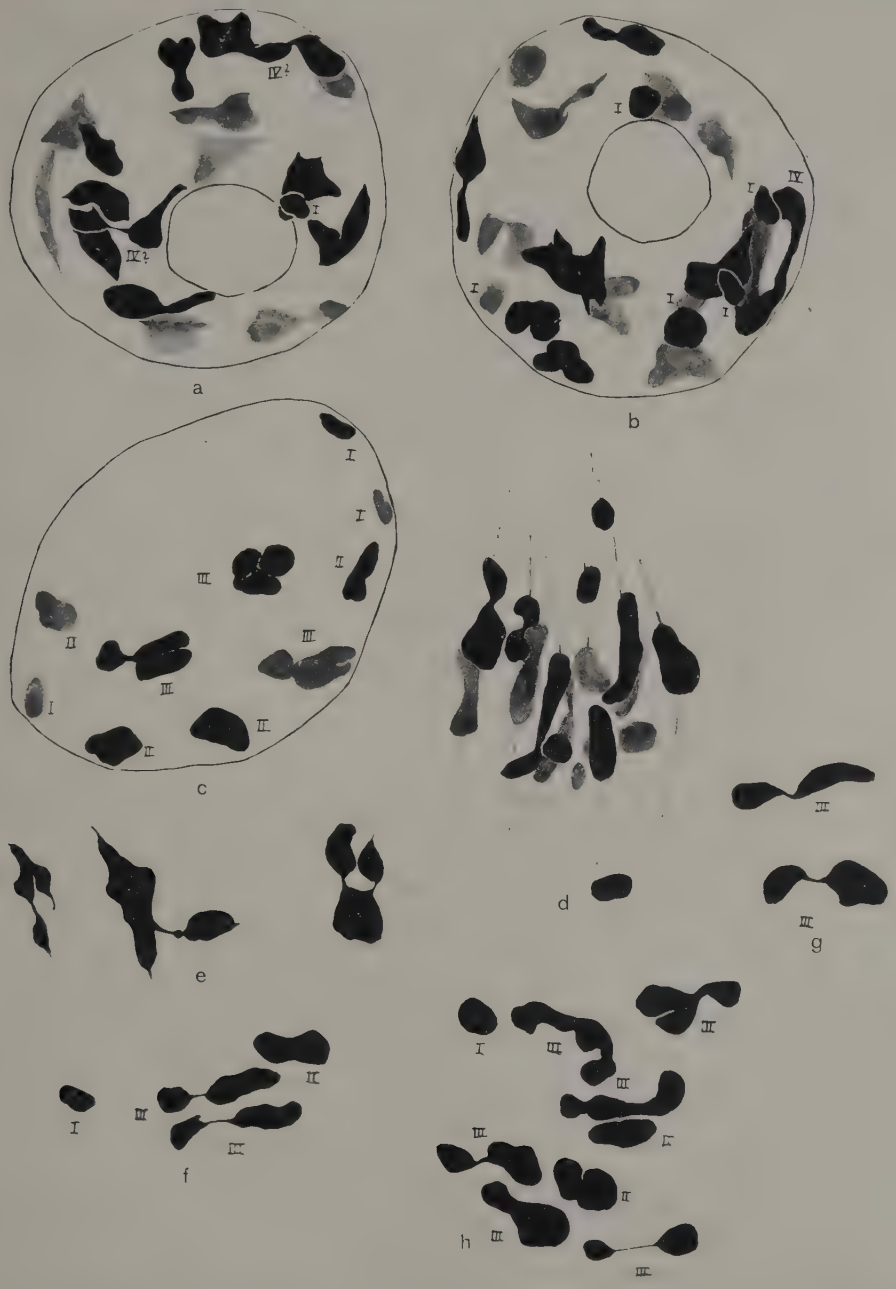


Fig. 4. RIBSTON. a diakinesis (A). — b diak. (C). — c diak. (detail.). — d metaphase I, tri-, bi- and univalents (not complete). — e metaphase I, one trivalent (left), two quadrivalents. — f—h metaphase I (tri-, bi- and univalents). — 5000 ×.

In the tetraploids of Pomoideae quadrivalents are not uncommon, the same as univalents and (more rare) sexivalents. Higher multivalents are also said to

Table IV. **Chromosome Conjugation in the Triploid Variety »Ribston» (diakinesis).**

Nucleus	*Quadrivalents*	*Trivalents*	*Bivalents*	*Univalents*	*Units* (total)
A (fig. 4 a) . . .	2	14	—	1	17
B	1	15	—	2	18
C (fig. 4 b) . . .	1	14	—	5	20
D	?	?	?	?	20
E	?	?	?	?	22
F	—	11	6	6	23
G	1	9	6	8	24
H	?	?	?	6	24
I	?	?	?	?	24

be identified (MOFFETT 1931), though their existence may appear doubtful, considering the obvious difficulty of interpretation.

Triploid pears, unlike triploid apples, show no multivalent associations higher than trivalents (MOFFETT 1934, table I.) The conjugation clearly points to the triploid pears being autotriploids, as are the triploid apples. The highest degree of trivalent formation found was $16_{III} + 1_{II} + 1_{I}$. The total number of units at metaphase I varied between 18 and 32, the most commen configurations lying between 20 and 26.

ROSCOE (1933, p. 161) recently gave some data on the triploid apple variety »Stark». At metaphase I of this variety 22—25 chromosome units are usually present, including varying numbers of quadrivalents, trivalents, bivalents and univalents. Some additional data have, finally, been presented by MIEDZYRZECKI (1933, 1934) on autotriploid apples and pears, and by SAX (1931, 1932) on allotriploid hybrids. SAX, however, appears not to have observed the possible existence of two types of triploids, as demonstrated by MOFFETT, and his discussions accordingly become irrevelant. His statement that »the number of lagging univalents at anaphase is undoubtedly a better index of chromosome association than counts at earlier stages» (SAX 1932, p. 364), is incorrect, as all univalents certainly do not lag at anaphase. The most reliable stage, with regard to univalents, appears to be diakinesis which has not been investigated by SAX.

My own contribution to the knowledge of the meiosis of triploids refers to the apple variety »Ribston». This variety has been repeatedly stated to be triploid ($3n = 51$), and as regards the material investigated by myself, its triploidy has been exactly corroborated by countings at anaphase I. Table IV gives some data on conjugation at diakinesis. As has been repeatedly stated, it is often difficult to distinguish with any degree of certainty beween the various kinds of bi- and multivalents in apples, and this circumstance is responsible for the scantiness of the data. The last column of the table gives the total number

of chromosome units in each nucleus. This number varies between 17 and 24, most of the nuclei lying between 20 and 24. In order to classify the units the number of univalents is first determined, as the univalents generally stand out clearly from the rest of the chromosome set. As is seen, univalents occur regularly, though they are not numerous. Quadrivalents are rare and may be difficult to recognize. Bivalents and trivalents are not easily distinguished from one another, and the proportion between these two classes has, therefore, been computed from the total number of units and the number of univalents and quadrivalents (the somatic number being 51). Multivalents of a higher order than quadrivalents have not been met with, at least not with any degree of certainty. On the whole, the conjugation is rather complete, closely agreeing with the type of autotriploidy, as already stated by MOFFETT and ROSCOE. In fig. 4 various types of chromosomes at diakinesis and metaphase I are represented.

Table V. **Number of Pollen Grains in Tetrads of »Ribston».**

Number of grains . . .	4	5	6	7
Tetrads counted	53	12	3	2

Table V contains a few countings of the number of grains in pollen tetrads of »Ribston». As is seen, the great majority of tetrads are morphologically quite normal, and the dwarf grains, when occurring, are always few. More than three extra grains in a tetrad have not been met with. Consequently, the dwarf grains may be calculated as not exceeding 10 % of the mature pollen. A sample of mature pollen of »Ribston», examined by myself some years ago, did really not contain more than about 13 % of abortive dwarf grains, which is in good consonance with the above result. The pollen of »Ribston» on the whole presents a (morphologically) normal appearance, though, as is well-known, its power of germination is not great. Other triploids may contain much higher percentages of abortive pollen (conf. below p. 168).

Another noteworthy fact is the absence of pollen dyads. In this apple variety I have, in fact, not found a single case, though I have repeatedly searched for dyads. If they do occur, they must be very rare. Therefore, unreduced pollen grains rarely, if ever, should be formed. This is obviously the reason why the offspring of triploid apples does not contain any polyploid plants — as might have been expected — but only aneuploids (MOFFETT 1931, NEBEL 1933). The conclusion might also be drawn that our triploid garden varieties have originated by fertilization of incidental, unreduced embryo-sacs with normal haploid pollen. Unreduced embryo-sacs may probably arise through apospory, as was recently shown by GORCZYŃSKY (1934), and the fertilization of such embryo-sacs is perhaps not excluded (conf. BERGMAN 1935, p. 180, on *Leontodon*; conf. also LILJEFORS 1934 on *Sorbus*).

C. Pollen Lethality.

1. Viability and Lethality in Diploids.

Through the investigations, briefly reviewed in the introduction, three main causes of pollen lethality in apples may be conceived: triploidy, influence of extreme temperatures and special pollen lethals. In an earlier paper, the writer (HEILBORN 1932) made a summary of the phenomena coming under this head, and presented some preliminary data regarding the theory of pollen lethals. In the present chapter a full account of pollen abortion in diploid apples is presented, the influence of temperature and of triploidy being discussed later. The chromosome numbers of some of the varieties dealt with in this chapter have not yet been determined, but the character of the pollen does not leave the slightest doubt as to the diploidy of these varieties.

The writer, gathering data on the percentage of pollen viability, i. e. the percentage of living pollen (in contradistinction to dead and empty grains), soon realized the necessity of obtaining not only mean values but also a picture of the variability within each variety. In order to secure this, the following working system was adopted. Each flower was regarded as a physiological unity and has, accordingly, been investigated and recorded separately, the number of pollen grains counted in a flower generally amounting to about 600—800 (sometimes less, sometimes much more). Flowers to be investigated are collected in the afternoon on the day before their opening and placed in water in a sunny window. Anthers already dehisced are carefully removed. The following morning pollen from freshly dehisced anthers is sown in a pendant drop of 10 % cane sugar solution in a moist chamber. After some hours (not more than 24) the cover glass with the drop is removed to a slide where, previously, a tiny drop of glycerine has been placed. There the drop of sugar solution rapidly spreads into a very thin layer, at the same time mixing with the glycerine. The pollen grains and tubes at first become plasmolyzed by the glycerine, but this plasmolyzation very soon disappears. The slides are now ready for investigation and may be kept for months without any visible change. This method of germination of the pollen grains before determining the viability percentage enables a simultaneous estimation of the power of germination of the (morphologically) normal and viable grains. As a rule, the percentage of germination seems to be only slightly lower than the percentage of viability. The percentages of viability are determined by counting a number of parallel bands more or less regularly distributed over the slides.

The differences between viable and abortive grains are shown in fig. 5. — The results of counting 359 flowers and more than 200,000 pollen grains from 31 diploid varieties during the five years 1930—1934 are presented in table VI, and all the percentage figures of this table have been plotted in the graph of fig. 6. In the table and the graph only such figures are included as have been obtained from apple trees in Hortus Bergianus at open-air temperatures (the values of »Hampus» are from a tree at Djursholm). Some results from special

experiments with high temperatures are dealt with further on. In my previous paper (l. c. 1932) I tried to divide the diploid apple varieties into distinct »viability classes» with regard to their contents of viable pollen grains. This classification has turned out to be more difficult than originally expected. In order to avoid arbitrariness in arranging the material the varieties have, therefore, been ranged in a falling sequence according to the mean values of table VI,

Fig. 5. Pollen of diploids. a Yellow Richard. — b Öland's Kungsäpple. — c Charlamovsky. — d Wealthy. — e Cellini. — f Gyllenkrok's Astrachan. — g Eneroth's Klaräpple.

and the values of the individual flowers have then been plotted separately. As is seen, there is, by this arrangement, an almost continuous transition between the presumptive viability classes, but any artificial division into distinct classes is, on the other hand, avoided.

It was soon found that one reason why the lines of distinction between the presumptive viability classes have been wiped out in such a way lies in the sensitiveness of the pollen to high temperatures. Special experiments (conf. fig. 8) have shown that the percentages of viability of »White Astrachan» and, to some

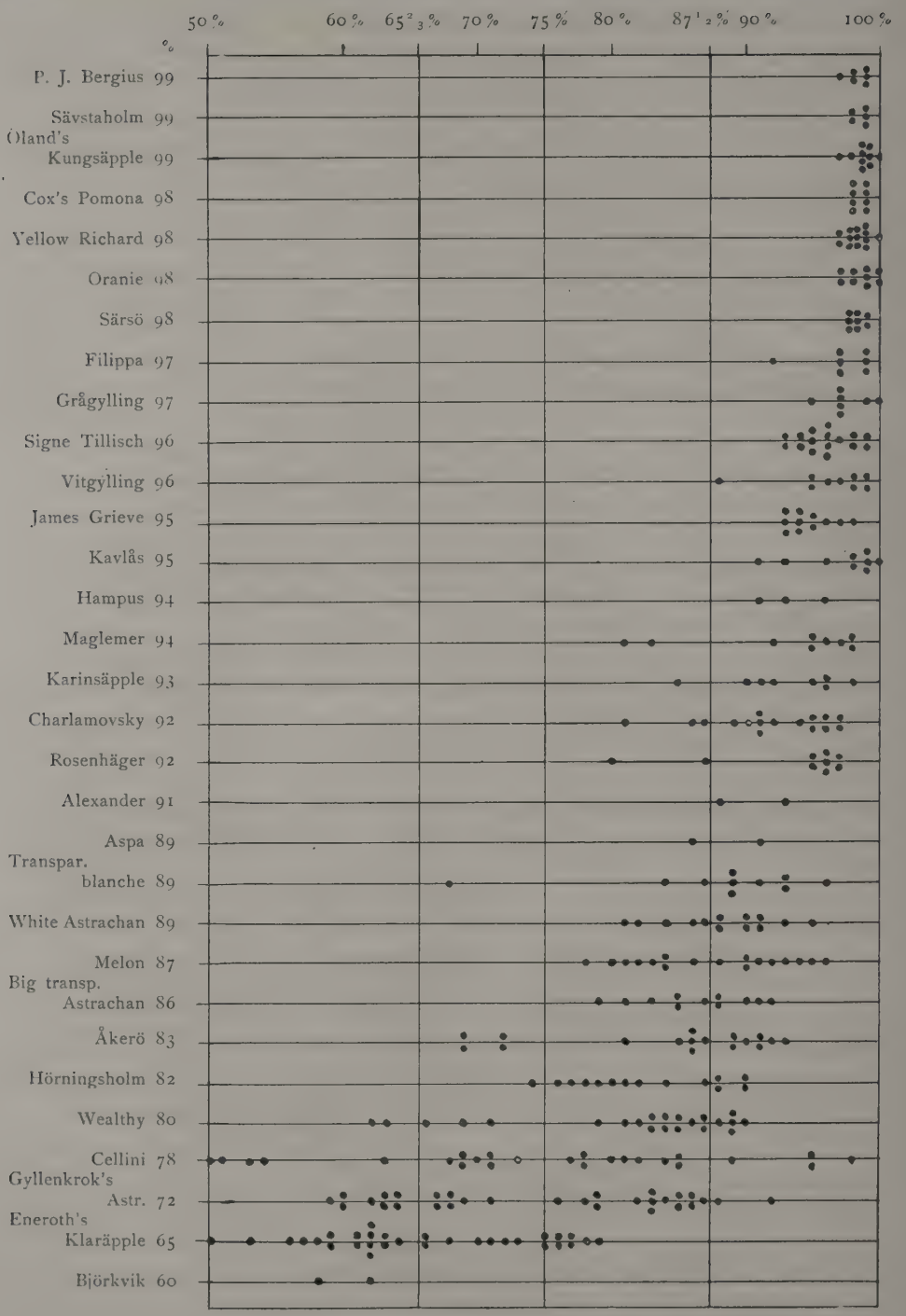


Fig. 6. Pollen viability percentages (table VI).

Table VI. **Pollen Viability Percentages of Diploid Apples.**
(Open-air temperature.)

Each determination refers to a single flower.

Åkerö.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%	Good	Empty	%
			561	91	86	955	142	87	498	226	69	454	71	86
			267	45	86	470	84	85	383	176	69	299	38	89
									444	170	72	360	28	93
									643	148	81	413	52	89
									583	227	72	362	39	90
												319	31	91
												616	53	92
												395	40	91

Total 8,022 good and 1,661 empty = 83 % good grains.

Alexander.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	—	—	—	—	—	—	—	—	—
			462	35	93									
			236	31	88									

Total 698 good and 66 empty = 91 % good grains.

Aspa.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	—	—	—
						881	86	91						
						524	83	86						

Total 1,405 good and 169 empty = 89 % good grains.

Astrachan, big transparent.

1930		1931			1932			1933			1934		
—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%	Good	Empty	%
		200	31	87	347	60	85	300	64	83	361	25	94
		364	99	79	462	106	81	266	47	85	349	26	93
								544	77	88	478	51	90
											399	53	88

Total 4,070 good and 639 empty = 86 % good grains.

Table VI (cont.).

Astrachan, Gyllenkrok's.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%
						866	361	71	346	238	59	436	58	88
						379	235	62	436	287	60	402	72	85
						741	351	68	599	345	63	407	34	92
						624	281	69	390	232	63	248	39	86
									384	257	60	366	100	79
									381	191	67	398	66	86
									385	220	64	311	64	83
									386	179	68	439	82	84
									399	199	67	341	110	76
									376	216	64	341	68	83
												330	67	83
												426	75	85
												300	84	78
												398	59	87
												349	95	79
												270	61	82

Total 12,454 good and 4,726 empty = 72 % good grains.

Astrachan, white.

1930			1931			1932			1933			1934		
Good	Empty	%	Good	Empty	%	Good	Empty	%	—	—	—	Good	Empty	%
—	—	87	266	63	81	694	93	88				526	57	90
—	—	90	164	32	84	623	85	88				493	47	91
			217	47	82							442	35	93
			388	40	91									
			458	75	86									
			551	30	95									

Total[1] 4,822 good and 604 empty = 89 % good grains.

Björkvik.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	—	—	—	—	—	—	—	—	—
			245	148	62									
			272	194	58									

Total 517 good and 342 empty = 60 % good grains.

[1] Besides data from 1930.

Table VI (cont.).

Cellini.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%	Good	Empty	%
			235	186	56	826	211	80	605	275	69	509	92	85
			124	112	53	581	73	89				306	88	78
			235	136	63	738	39	95				330	76	81
			202	188	52	702	332	68				540	115	82
			240	110	69	582	161	78				540	104	84
			167	135	55	607	249	71						
			211	86	71	979	55	95						
			492	185	73	833	248	77						
			151	65	70	846	144	85						
						612	13	98						

Total 12,193 good and 3,478 empty = 78 % good grains.

Charlamovsky.

1930			1931			1932			1933			1934		
Good	Empty	%	Good	Empty	%	Good	Empty	%	—	—	—	Good	Empty	%
—	—	91	404	26	94	621	150	81				181	30	86
—	—	91	590	16	97	607	23	96				370	53	87
			527	63	89	696	78	90				701	34	95
						522	40	93				484	18	96
						457	26	95				483	47	91
												436	15	97

Total[1] 7,079 good and 619 empty = 92 % good grains.

Cox's Pomona.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	—	—	—	—	—	—	Good	Empty	%
			593	11	98							462	11	98
			341	5	99							429	6	99
												536	4	99
												394	8	98
												624	13	98
												384	4	99

Total 3,763 good and 62 empty = 98 % good grains.

[1] Besides data from 1930.

Table VI (cont.).

Eneroth's Klaräpple.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%
						523	434	55	424	255	62	204	128	61
						374	276	58	488	272	64	313	100	76
						725	450	62	459	177	72	310	105	75
						388	387	50	543	169	76	225	115	66
						244	183	57	446	264	63	280	74	79
						400	245	62	381	105	78	318	95	77
									447	162	73	180	115	61
									462	190	71	266	140	66
									441	212	68	284	97	75
									397	316	56	216	152	59
									471	144	77	165	116	59
									297	179	62	312	136	70
												210	126	63

Total 11,193 good and 5,919 empty = 65 % good grains.

Filippa.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	Good	Empty	%
						797	21	97				458	15	97
						843	25	97				466	40	92
												486	5	99
												375	2	99
												496	5	99

Total 3,921 good and 113 empty = 97 % good grains.

Grågylling.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	Good	Empty	%
						779	21	97				534	15	97
						758	44	95				398	4	99
												342	10	97
												282	8	97
												256	0	100

Total 3,349 good and 102 empty = 97 % good grains.

Table VI (cont.).

Hampus.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	—	—	—
						770	36	96						
						721	51	93						
						574	54	91						

Total 2,065 good and 141 empty = 94 % good grains.

Hörningsholm.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	—	—	—	Good	Empty	%	Good	Empty	%
									617	106	87	435	135	76
									671	88	88	406	111	79
									596	128	82	480	112	81
									605	83	88	347	40	90
									594	110	84	395	42	90
									617	218	74	378	104	78
									554	166	77			
									904	228	80			

Total 7,599 good and 1,671 empty = 82 % good grains.

James Grieve.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	—	—	—	—	—	—	Good	Empty	%
												562	43	93
												583	43	93
												406	23	95
												360	29	93
												627	43	94
												625	31	95
												535	35	94
												616	38	94
												516	13	98
												691	26	96
												639	23	97

Total 6,160 good and 347 empty = 95 % good grains.

Table VI (cont.).

Karinsäpple.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	—	—	—	Good	Empty	%	Good	Empty	%
									716	34	95	735	68	92
									1,031	39	96	584	25	96
									919	103	90	637	15	98
												453	79	85
												395	40	91

Total 5,470 good and 403 empty = 93 % good grains.

Kavlås.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%
						949	97	91	628	14	98	485	7	99
						1,345	99	93	422	6	99	429	9	98
									300	3	99	209	1	100
												525	22	96

Total 5,292 good and 258 empty = 95 % good grains.

Maglemer.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	Good	Empty	%
						909	15	98				347	70	83
						969	31	97				302	73	81
												353	29	92
												599	13	98
												588	33	95
												488	23	95
												433	16	96

Total 4,988 good and 303 empty = 94 % good grains.

Melon.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%	Good	Empty	%
			449	124	78	507	100	84	599	113	84	469	27	95
			751	169	82	413	95	81	477	97	83	397	41	91
									574	144	80	309	27	92
												376	51	88

Table VI (cont.).

Melon (cont.).

1930			1931			1932			1933			1934		
—	—	—	—	—	—	—	—	—	—	—	—	Good	Empty	%
												373	60	86
												457	51	90
												461	33	93
												443	20	96
												432	29	94
												435	46	90

Total 7,922 good and 1,227 empty = 87 % good grains.

Öland's Kungsäpple.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	Good	Empty	%
						755	14	98				613	3	100
						532	4	99				291	8	97
												369	5	99
												352	2	99
												624	4	99
												392	3	99
												797	10	99
												445	6	99

Total 5,170 good and 59 empty = 99 % good grains.

Oranie.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%	Good	Empty	%
			189	5	97	552	7	99	847	27	97	484	6	99
			181	0	100	545	12	98				546	11	98
			361	3	99									
			191	0	100									

Total 3,896 good and 71 empty = 98 % good grains.

P. J. Bergius.

1930			1931			1932			1933			1934		
Good	Empty	%	Good	Empty	%	Good	Empty	%	—	—	—	—	—	—
—	—	99	543	7	99	678	6	99						
—	—	97	321	6	98	614	11	98						

Total[1] 2,156 good and 30 empty = 99 % good grains.

[1] Besides data from 1930.

Table VI (cont.).

Rosenhäger.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	—	—	—	Good	Empty	%
						736	114	87				512	19	96
						655	164	80				577	22	96
						364	18	95				345	10	97
						911	39	96				571	20	97
						534	29	95						

Total 5,205 good and 435 empty = 92 % good grains.

Särsö.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%
						1,045	19	98	720	10	99	572	10	98
						410	8	98	609	7	99	441	7	98
												278	6	98
												448	9	98

Total 4,523 good and 76 empty = 98 % good grains.

Sävstaholm.

1930			1931			1932			1933			1934		
Good	Empty	%	—	—	—	Good	Empty	%	—	—	—	Good	Empty	%
—	—	98				948	20	98				392	2	99
						498	4	99				358	5	99

Total[1] 2,196 good and 31 empty = 99 % good grains.

Signe Tillisch.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	—	—	—	Good	Empty	%	Good	Empty	%
			293	4	99				731	31	96	408	11	97
			512	12	98				947	55	95	655	47	93
									715	42	94	546	38	93
												657	31	95
												665	17	98
												792	7	99
												733	31	96

[1] Besides data from 1930.

Table VI (cont.).

Signe Tillisch (cont.).

1930			1931			1932			1933			1934		
—	—	—	—	—	—	—	—	—	—	—	—	Good	Empty	%
												650	26	96
												527	25	95
												484	19	96
												650	43	94

Total 9,965 good and 439 empty = 96 % good grains.

Transparente Blanche.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%
						769	79	91	632	122	84	510	66	89
						1,131	51	96	399	49	89	323	38	89
												424	34	93
												413	60	87
												255	120	68
												459	37	93

Total 5,315 good and 656 empty = 89 % good grains.

Wealthy.

1930			1931			1932			1933			1934		
—	—	—	—	—	—	Good	Empty	%	Good	Empty	%	Good	Empty	%
						1,067	181	85	632	261	71	392	58	87
						712	84	89	501	303	62	420	91	82
									358	213	63	279	72	79
									431	191	69	400	66	86
									366	192	66	382	78	83
												312	34	90
												419	82	84
												332	39	89
												328	67	83
												316	74	81
												558	68	89
												312	59	84
												439	80	85
												608	91	87
												312	41	88

Total 9,876 good and 2,425 empty = 80 % good grains.

Table VI (cont.).

Vitgylling.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	—	—	—	Good	Empty	%	Good	Empty	%
			408	5	99				396	56	88	451	11	98
			335	6	98				881	33	96	449	6	99
									906	43	95	356	12	97
									582	32	95			

Total 4,764 good and 204 empty = 96 % good grains.

Yellow Richard.

1930			1931			1932			1933			1934		
—	—	—	Good	Empty	%	Good	Empty	%	—	—	—	Good	Empty	%
			594	7	99	997	18	98				286	1	100
			775	17	98	636	18	97				379	10	97
						363	6	98				286	7	98
												363	6	98
												376	2	99
												322	4	99
												557	6	99
												373	6	98

Total 6,307 good and 108 empty = 98 % good grains.

Grand total[1] 199,739 pollen grains counted.

extent, »Oranie», are decreased by a greenhouse temperature of +25°—30° C., while »Cellini» is not influenced by such a moderate increase in temperature. In a similar way, it has been noted that a rather exceptional heat during the time of flowering of the apples in 1933 rather seriously decreased the pollen viability of several varieties (conf. below fig. 9). Finally, the data on some of the varieties are too scanty to permit of safe conclusions. In view of all these circumstances I have, therefore, made certain corrections in order to avoid, as far as possible, the different influence of temperature in different years, as well as mere chance effects. All data from 1933 have, accordingly, been omitted, except the single percentage figure for »Cellini», as this variety has proved insensitive to an increase of temperature. For this last-mentioned reason, the numerous greenhouse values for »Cellini» have, instead, been added. Finally, the data for »Hampus», »Alexander», »Aspa» and »Björkvik» have been omitted. The sequence of the varieties has, however, not been altered! The corrected survey is presented in fig. 7.

[1] Besides data from 1930.

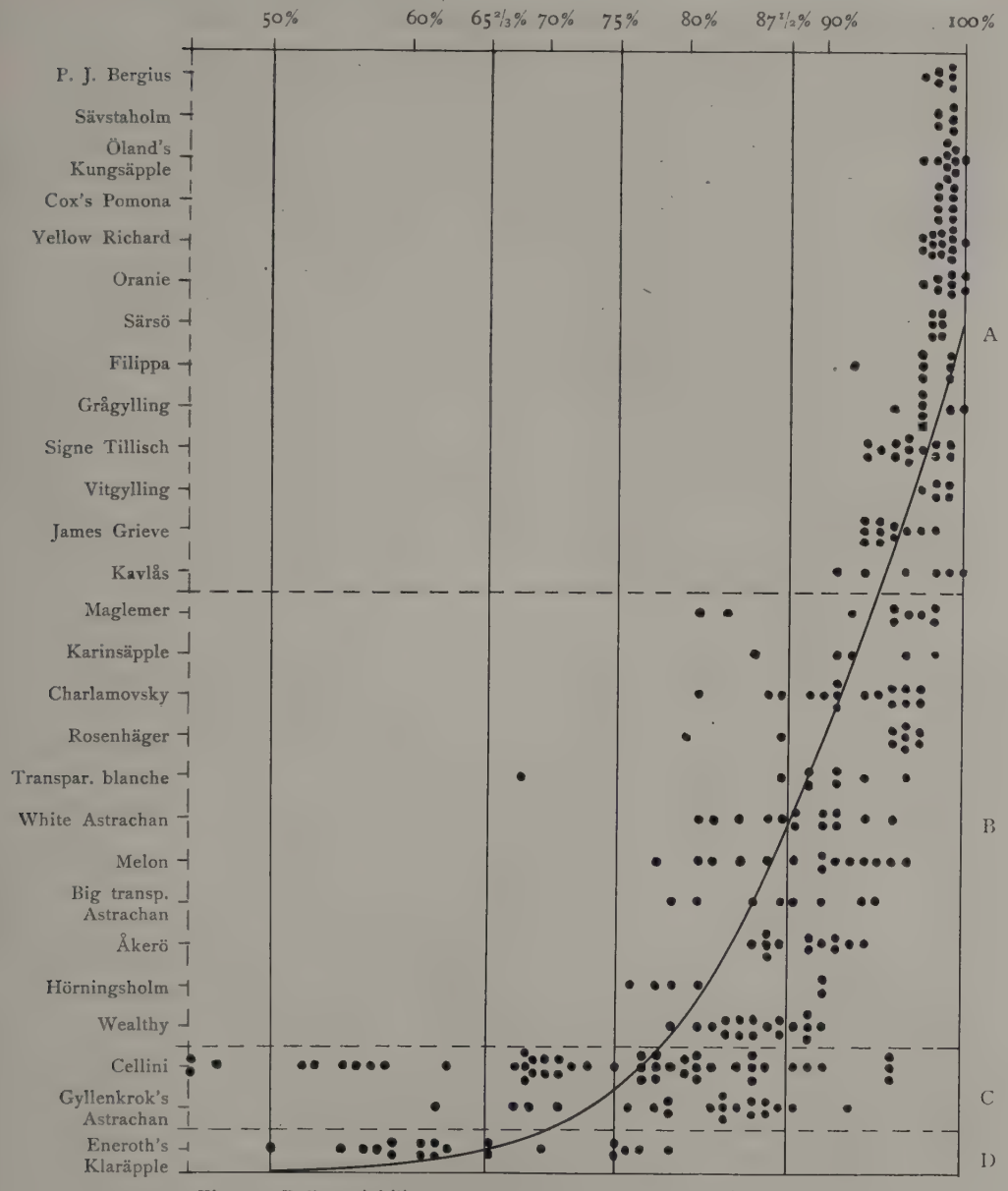


Fig. 7. Pollen viability percentages, corrected survey (conf. text).

The graph of fig. 7 gives better information as to the existence of distinct viability classes. Numerous varieties adhere strongly to the line of 100 % of viable pollen and constitute a class (*A*) of wholly fertile apples. The percentage values of numerous other varieties group themselves rather regularly around a value which may correspond to a theoretical mean of 7/8th or 87 ½ % of good pollen and 1/8th dead (class *B*). Two or three varieties differ sharply from the

rest. Of these »Cellini» is remarkable not only for its mean value — 3/4th or 75 % of good pollen and 1/4th dead — but also for the wide dispersion of the individual values, conspicuously differing from class *B*. The counting of more than 27,000 pollen grains from four different trees of this variety gave a pollen viability of 77 % — very close to the theoretical value 75. This percentage of viability — which has proved practically independent of temperature, and should be regarded as reliable — and the remarkable dispersion of the values of the individual flowers tend to show, that the mechanism underlying the lethality is here different from the corresponding mechanism in class *B*, and place »Cellini» in a class of its own (class *C*). It is possible, though scarcely probable, that »Gyllenkrok's Astrachan» likewise belongs to this class (it may, instead, belong to class *B*). »Eneroth's Klaräpple», finally, represents a still further viability class with a mean that closely approaches a theoretical value of 21/32nds or about 65 2/3 % of viable pollen.

As is seen, there are two great — and equally great — classes of apple varieties (*A* and *B*), and two small and likewise equal classes (*C* and *D*). The theoretical means of the four classes have been united with a curve and the various swarms of individual determinations are seen to group themselves very regularly along this curve. The interpretation of these theoretical values will be presented later.

As to pears, definite evidence on abortive pollen is wanting. There are only a few preliminary data to be found in the recent works by MIEDZYRZECKI (1933, 1934). According to this author various percentages of »micro-grains» (from 10 to 20 %) are found in a number of diploid varieties investigated. It seems probable, therefore, that these varieties belong to a viability class corresponding to class *B* among apples. Further investigations are necessary.

2. Influence of high Temperatures.

The writer's earlier researches into apple cytology (HEILBORN 1928, 1930) have shown that the chromosome conjugation and the spindle formation at meiosis may be disturbed by high temperatures, but that this injurious effect is scarcely perceptible below + 30° C. In Swedish gardens this type of sensitiveness to excessive heat can have no practical importance. From Morocco, however, MIEDZYRZECKI (1933, 1934) reports cases where excessively high temperatures during February and March 1933 had caused irregularities in the meiosis of some diploid apples. These irregularities entailed a notable increase in pollen sterility. In subtropical gardens such effects may be of importance, in Swedish gardens they are practically negligible.

High temperatures may, however, act injuriously in another way. Such is the conclusion that may be drawn from two series of observations to be presented below.

In my paper of 1932 (l. c. p. 2) reference was briefly made to a series of experiments with dwarf apple trees which had been kept alternately at hot

greenhouse and at open-air temperature in Hortus Bergianus. The varieties thus investigated are »Oranie», »White Astrachan» and »Cellini». In the course of this the pollen viability percentages of the flowers and the chromosome conjugation at meiosis have been continuously determined. It was soon found that the reduction division, and especially the chromosome conjugation, was not injured or changed by the high temperature in the greenhouse (not exceeding + 25°—30° C.). Data obtained on chromosome conjugation have, therefore, been included in Table II of the present work, and the meiotic phenomena have been regarded as insignificant for the interpretation of pollen sterility in diploid apples.

On the other hand, the viability percentages of the greenhouse material showed a clear effect of the rise in temperature, nota bene in two of the varieties (»Oranie» and »White Astrachan») but not in the third (»Cellini»). From all this the conclusion should be drawn that the post-meiotic ripening, before the opening of the flowers, is the most sensitive period in the development of the pollen, and that a strong increase in temperature during that period may manifestly increase the lethality of the pollen.

The experiments were carried out in the following way. In the spring of 1930 one dwarf tree of »Oranie», one of »White Astrachan» and two of »Cellini»

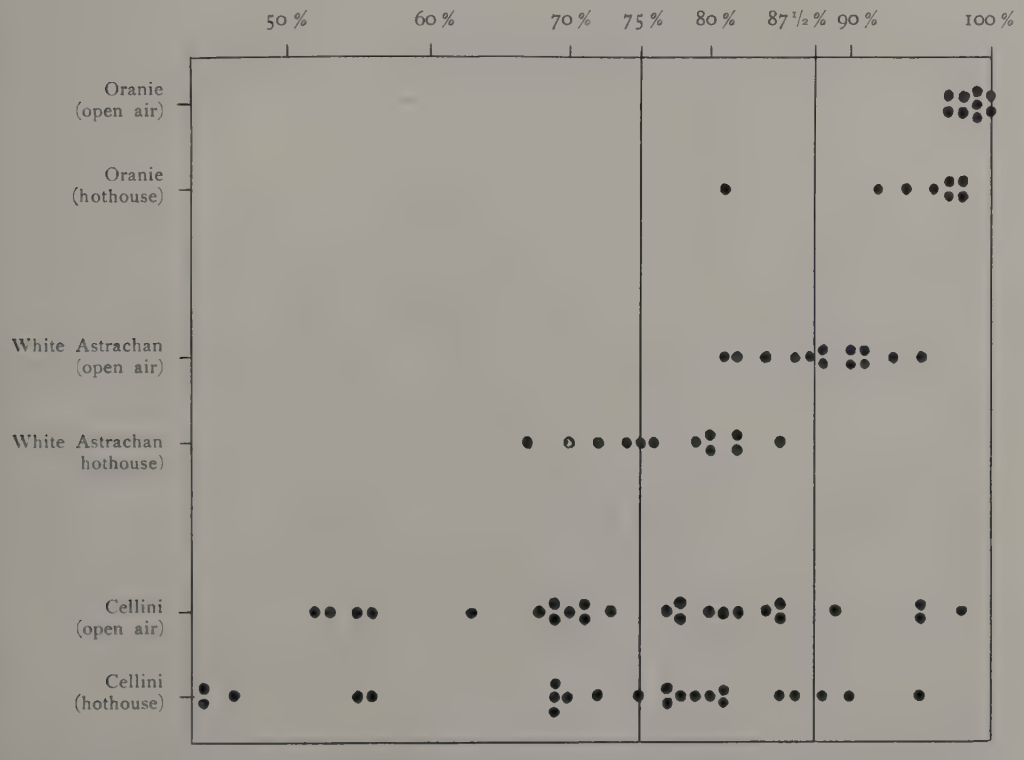


Fig. 8. Influence of high temperature on pollen viability percentages. Hothouse experiments. Each determination represents one flower.

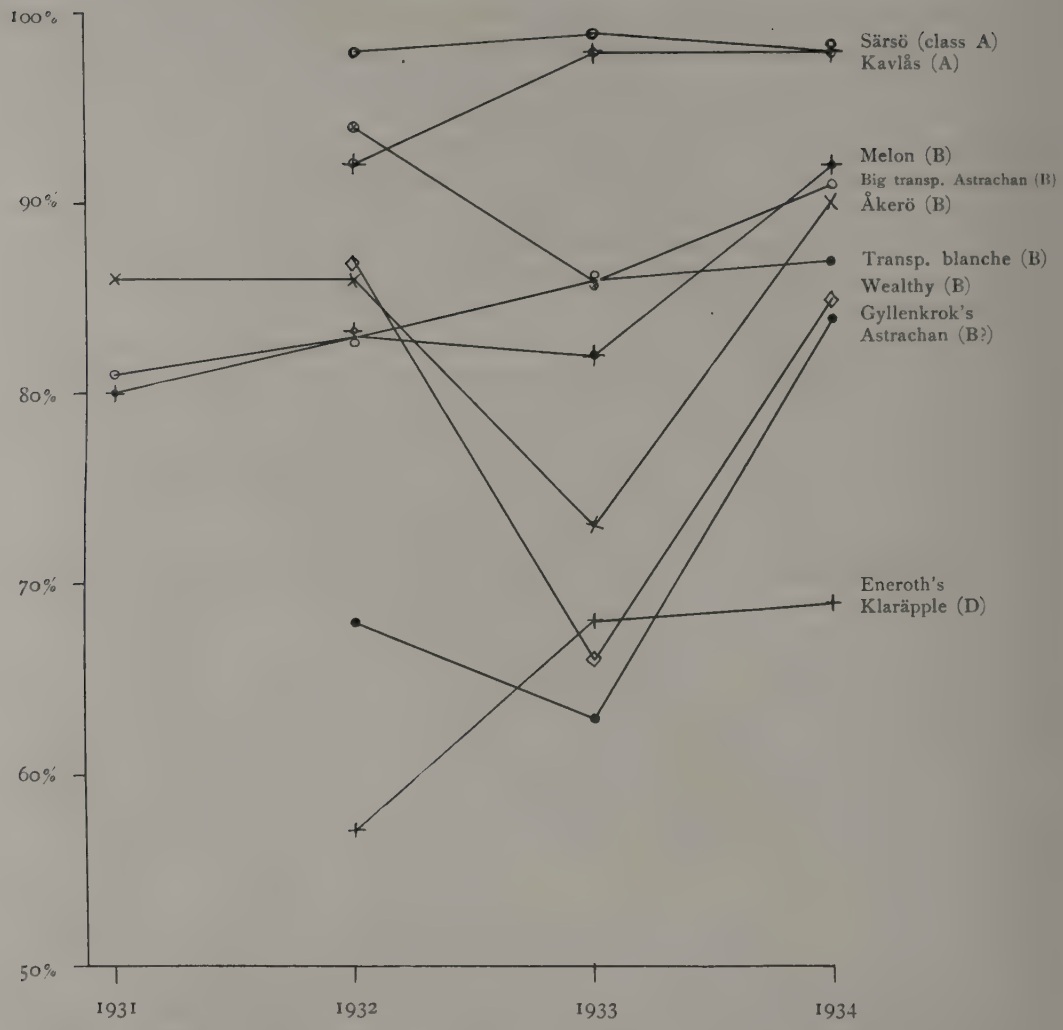


Fig. 9. Pollen viability: comparison between different years. Mean values at open air temperature.

were placed in one of the heated greenhouses of Hortus Bergianus and the meiotic divisions in the pollen mother-cells as well as the pollen viability in the ripe anthers were accordingly investigated. The trees were then transplanted into the open air and investigated there in the spring of 1931. New greenhouse data were obtained from »Oranie» and »Cellini» in 1932, and from »White Astrachan» and »Cellini» in 1933. Finally, in 1933, a single viability determination on »Oranie» and one on »Cellini» were obtained at open-air temperature. All the data on pollen viability, thus obtained, have been plotted in the graph of fig. 8. To these have, further, been added all open-air data obtained from other trees than the above-mentioned dwarf trees (included in table VI). A glance at the graph immediately shows that the viability per-

centages of »Oranie» and »White Astrachan» have been visibly depressed by the hothouse treatment, whereas »Cellini» does not show any effect of this treatment. On account of this the hothouse data of »Cellini» have been included in the graph of fig. 7 (conf. above).

The second series of observations on the influence of high temperature on pollen viability is as follows. In the neighbourhood of Stockholm apple trees generally pass the stage of reduction division in the pollen mother-cells in the beginning and middle of May, and enter their time of flowering about the first of June. In 1931 and 1932 nothing extraordinary was noted in the course of the work, but in 1933 a heat wave occurred during the apple flowering in June, and in 1934 rather abnormal heat was noted during the period of reduction division in May. Now, the viability percentages, determined in 1931, 1932 and 1934 appear more or less normal, whereas most of the viability figures for 1933 are clearly depressed (conf. the graphs of fig. 9). This must be interpreted to the effect that the last post-meiotic stages during the ripening of the pollen is a sensitive period, and that the hot weather during that period in 1933 had an injurious effect. On account of this all viability values of 1933 have been omitted from the graph of fig. 7 (conf. above p. 160). The hot weather during meiosis in 1934, on the other hand, had no visible effect on pollen viability.

From these experiments and observations it may be concluded that high temperatures may have a depressing effect upon the pollen viability of apples, and that a part of the conspicuous variability, so often observed in pollen sterility of diploid apples, may be the result of variations in temperature.

3. Factorial Interpretation.

A factorial interpretation of the pollen lethality in apples was tentatively suggested in my paper 1932 (l. c. p. 12). This interpretation was founded on the preliminary viability figures then available; on the often observed variability in pollen sterility which renders the classification into viability classes more or less difficult; on the theory of »secondary polyploidy» presented by DARLINGTON and MOFFETT (1930); and, finally, on the presumptive occurrence of numerous multivalents in diploid apples reported by the last-mentioned authors. On these premises the pollen lethality of diploid apples was interpreted as resulting from the interaction of polymeric recessive pollen lethals, and the above-mentioned variability was regarded as a genetic effect of the formation of multivalent chromosomes. Now, multivalent chromosomes have later proved to be so exceedingly rare that they can have no effect whatever upon pollen lethality. At the same time, increasing material has enabled a more accurate analysis of pollen viability. Hence, my first interpretation has been changed in some details. The new and modified factorial scheme is presented below.

According to DARLINGTON and MOFFETT (1930) the haploid chromosome set of apples has the constitution *AABBBCCCDDEEFFGG* and the so called diploid apples are in reality trebly hexasomic tetraploids. Their chromosome

conjugation shows them to be allotetraploids. Now, I suppose one system of pollen lethals to be situated in the group of *A*-chromosomes and another system of such lethals in the group of *D*-chromosomes. If we let the letters signify genes instead of chromosomes, then we suppose every pollen grain to die which has received all three recessive genes of the *A*-system. The constitution of such an abortive pollen grain is thus *aaaBBBCCCDDEEFFGG*. In a similar way, all pollen grains *AAABBBCCCddEEFFGG* also die and shrivel. With reference to the allotetraploidy of the apples the lethal combinations should rather be signed $a_1a_2a_3$ and d_1d_2.

These two systems of recessive lethals will act in the following way. All apple varieties of our gardens are probably hybrids originated by intra-specific crossing. The recessive lethals now characterizing many of these varieties must, consequently, have been transmitted from the parental specimens through the uniting gametes. These gametes may have been $A_1A_2A_3$, $a_1A_2A_3$, $A_1a_2A_3$, $A_1A_2a_3$, $a_1a_2A_3$, $a_1A_2a_3$, or $A_1a_2a_3$ but not $a_1a_2a_3$ (this last-mentioned being the lethal combination). They may also have been D_1D_2, d_2D_2 or D_1d_2, but not d_1d_2. On account of the allotetraploidy a chromosome A_1 always conjugates with A_1, A_2 with A_2, and A_3 with A_3, D_1 with D_1, and D_2 with D_2. Hence, out of all possible combinations of the viable gametes enumerated above, only the following will show partial pollen lethality:

$\frac{a_1a_2A_3}{A_1A_2a_3}$, $\frac{a_1A_2a_3}{A_1a_2A_3}$ and $\frac{A_1a_2a_3}{a_1A_2A_3}$ — which are all 1/8th-sterile — as well as $\frac{a_1a_2A_3}{a_1A_2a_3}$, $\frac{a_1a_2A_3}{A_1a_2a_3}$, $\frac{a_1A_2a_3}{A_1a_2a_3}$ and $\frac{d_1D_2}{D_1d_2}$ — which are all 1/4th-sterile. All other combinations are wholly fertile!

According to this interpretation the 1/4th-sterile varieties should be about as numerous as the 1/8th-sterile. This is not the case. We suppose, therefore, that all the 1/4th-sterile types resulting from the combination of *Aaa*-gametes inter se suffer from zygotic weakness or, maybe, lethality. Such combinations probably die as very young plants, or, perhaps, in an embryonic stage. Also, the male gametes *Aaa* themselves may very well be semi-lethal. It is at least not improbable that the injurious effect of high temperatures may depend upon the dying off of just such gametes, and *Aaa* pollen tubes may, further, become eliminated through selective pollen tube growth. The very few 1/4th-sterile varieties actually existing therefore probably owe their sterility to the *d*-genes exclusively.

As a consequence of two independent systems of pollen lethals — the *A*-system and the *D*-system — apple varieties combining both must also be assumed to exist. Such varieties should have a pollen viability of $^7/_8 \cdot {}^3/_4 = {}^{21}/_{32} =$ about 65 2/3 %. One such variety has really been found and may be regarded as a proof of the existence of two independent systems of lethals.

If 1/4th-steriles of the constitution $a_1a_1A_2a_2A_3a_3$ and similar types also exist, a still further type of combination might be predicted, having a pollen viability of $^3/_4 \cdot {}^3/_4 = {}^9/_{16} = 56\ {}^1/_4$ %. But no such variety has yet been found.

Summarizing the above discussion we thus arrive at the following results.

There is one group of wholly fertile varieties (class *A*). In the haploid group of such a variety never more than two of the three pollen lethals a_1, a_2 and a_3 are contained. Varieties of class *A* may be $A_1A_1A_2A_2A_3A_3D_1D_1D_2D_2$, $a_1a_1A_2a_2$ $A_3A_3D_1d_1D_2D_2$ a. s. o. — There is, further, a group of $^1/_8$th-sterile varieties (class *B*, showing $^7/_8$th viability). These varieties are heterozygous as regards all three gene pairs of the *A*-system. They are, consequently, $A_1a_1A_2a_2A_3a_3$, and at meiosis the following types of pollen are formed: $AAA+3AAa+3Aaa+aaa$ ($^7/_8$th viability). Varieties of class *B* may contain one of the two recessives d_1 or d_2, but not both. — The third group contains $^1/_4$th-sterile varieties (class *C*, showing $^3/_4$th viability). These varieties are heterozygous as regards both gene pairs of the *D*-system. They are $D_1d_1D_2d_2$ and at meiosis the following types of pollen are formed: $DD+2Dd+dd$ ($^3/_4$th viability). Varieties of this type may be $A_1a_1A_2A_2A_3A_3D_1d_1D_2d_2$, $A_1A_1A_2a_2$ $A_3a_3D_1d_1D_2d_2$ a. s. o. — Varieties of class *D*, finally, must be $A_1a_1A_2a_2A_3a_3D_1d_1D_2d_2$. At meiosis the following types of pollen are formed: $AAADD+2AAADd+3AAaDD+6AAaDd+3AaaDD+6AaaDd+AAAdd+3AAadd+3Aaadd+aaaDD+2aaaDd+aaadd$. Of these combinations the 21 first are viable while the remaining 11 die ($^{21}/_{32}$nd viability).

If *a*-lethals are common and *d*-lethals are rare, it is easily seen that classes *A* and *B* must both be great; and classes *C* and *D* both small (conf. the graph of fig. 7).

The variability among the percentage figures of table VI and the accompanying graphs is partly due to pure chance, and the lack of distinction between the various viability classes is merely the result of transgressive variability, the mean values lying too near one another to be quite sharply distinguished (65 $^2/_3$, 75, 87 $^1/_2$ and 100 %). The great dispersion of the viability values of »Cellini», as compared with varieties of class *B*, may be due to some disturbance in the chance distribution within the chromosome pairs D_1d_1 and D_2d_2. Such a disturbance might, perhaps, be an outcome of a very pronounced secondary association between these gemini. One might suggest the secondary association to be stronger within the *D*-group than within the *A*-group.

The differences in the influence of high temperature on different apple varieties should, finally, be referred to differences in genotypic constitution. With regard to pollen lethals, we may venture the following suggestions (conf. the graphs of figs. 8 and 9). Varieties of class *A* appear to be, generally, only slightly sensitive to high temperatures. They may be imagined as more or less homozygous with regard to the dominant *A*-genes, only rarely having such a constitution as to give rise to a considerable percentage of pollen of the semi-lethal *Aaa*-type. All the varieties of class *B*, again, have such a constitution as to produce a pollen of which $^1/_8$th aborts and $^3/_8$ths or 37 $^1/_2$ % is of the semi-lethal type *Aaa*. The data so far available are in good consonance with this, because all the *B*-varieties included in the graphs of fig. 8 and 9 appear to be decidedly sensitive to high temperatures. The variety »Cellini», finally, should be regarded as homozygous, or nearly so, with regard to the dominant *A*-genes, as it has been found to be practically insensitive to high temperatures.

It may well have the constitution $A_1a_1A_2A_2A_3A_3D_1d_1D_2d_2$, or something very similar. Of »Eneroth's Klaräpple» no very conclusive data are available.

The above interpretation of partial pollen lethality and of sensitiveness to high temperatures is an application of the modern conception of quantitative gene effects. The constitution *aaa* is regarded as leading to pollen abortion, and *Aaa* to semi-lethality, whereas *AAa* and *AAA* give healthy and viable grains; *dd* are abortive; *Dd* and *DD* are viable. This is in good agreement with general experiences of polymeric genes.

Some further aspects of these polymeric lethals will be discussed later.

4. Viability in Triploids.

The pollen germination tables of earlier authors show the germination percentages of triploid apples to vary between 4 and 34 (the diploids lying between 46 and 98), whereas the few data available on empty pollen grains among triploids suggest a variation between 15 and 94 % (conf. HEILBORN 1932, p. 15 and table 2, p. 10). There is generally a considerable portion of the pollen of

Table VII. **Percentages of living Pollen Grains in triploid Apples.**

Each determination refers to a single flower.
(Open-air temperature)

Variety		*Pollen grains*			
		Living	*Empty*	*% living*	*% (total)*
Hornsberg (3n?) (1932)		1,075	241	82	
		878	216	80	
	Total				81
Hurlbut (1932)		390	50	89	
		220	76	74	
	Total				83
Ribston (1931)		172	25	87	
	Total				87
Rossvik (1932, 1933)		289	150	66	
		355	239	60	
		206	127	62	
		293	187	61	
		513	340	60	
	Total				61

triploid apples which is viable, but does not easily germinate. This peculiarity is due to chromosome aberrations during meiosis. A part of the dead grains of triploids, especially the dwarf grains, likewise owe their existence to chromosome irregularities. It is, however, a noteworthy fact that obviously there exist great differences between different triploid varieties with regard to the percentage of dead grains. Table VII contains some countings.

To the figures of table VII may be added a few data from earlier works cited in my previous paper (l. c. 1932, p. 10). According to these last-mentioned data »Blenheim Orange» has 84 % of living pollen, »Jaques Lebel» 50 %, »Schöner von Boskoop» 47 %, and »Gravensteiner» 6 %. Of the eight triploid varieties

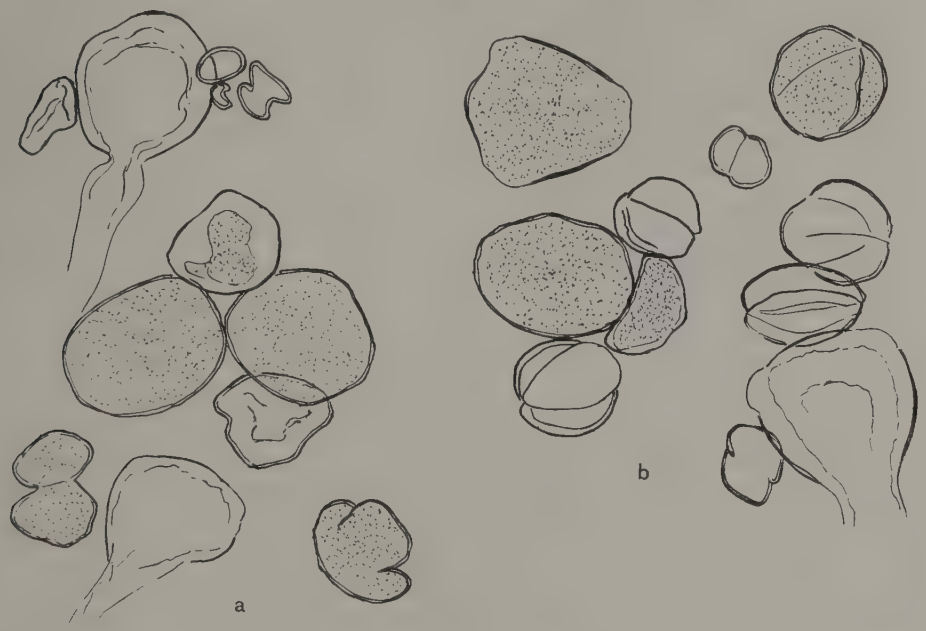


Fig. 10. Pollen of triploids. a Frösåker. — b Rossvik.

thus investigated four have 81—87 % of living pollen grains, three have 47—61 %, and one has only 6 %. These groups probably correspond to the viability classes among diploid apples. The first group (81—87 %) may correspond to class *A*, most of the abortive pollen grains here originating from the micro-grains of the irregular pollen tetrads. These abortive grains owe their origin to chromosome aberrations exclusively (conf. above p. 147). The group 47—61 %, again, probably represents triploids of type *B* where a part of the pollen abortion is caused by irregularities at meiosis, the remaining part being due to pollen lethals. To this group probably also belong two triploids which according to MIEDZYRZECKI (1933, 1934) have 27 resp. 36 % »micro-grains» (73 resp. 64 % living grains). »Gravensteiner» obviously corresponds to either class *C* or class *D*. As is seen, the systems of pollen lethals acting among the diploid apples seem to reappear in a similar way among the triploids.

D. Polyploidy.

1. The Theory.

DARLINGTON and MOFFETT (1930) advanced the hypothesis that the so called diploid apples are, in reality, polysomic tetraploids, having the constitution *AAABBBCCCDDEEFFGG* and descending from diploid ancestors, with a haploid set of the following 7 chromosomes: *ABCDEFG*. The authors, however, present their hypothesis in such a way as to leave the reader in doubt regarding several details. This has caused some confusion in the discussion following upon the publication of their work.

Thus SAX in two papers (1931, 1932) has criticized the theory rather severely. He argues: (1) that the numerous quadrivalents and sexivalents originally reported must have a disturbing effect upon the stability of the secondarily balanced polyploids (an effect not observed, however); (2) that the secondary association (»clumping of chromosomes») may be of little significance with regard to the presumed polyploidy (this view is, however, contrary to the new results of several recent authors); (3) that DARLINGTON's and MOFFETT's analysis of the progeny from a triploid apple pollinated with a diploid refers to statistically quite insufficient material (this is, indeed, true, but recent results by NEBEL fully agree with the above authors'; conf. below); (4) that DARLINGTON's and MOFFETT's description of their results indicate an autopolyploid condition, while the cytological studies of SAX himself and of other authors make an allopolyploid interpretation more likely. The basic number 8 is, furthermore, regarded as more likely (why?), or the Pomoideae might be looked upon as allotetraploids descending from one parent with 8 and another with 9 chromosomes [$2\times(8+9)=34$]. No doubt, modern studies in polyploidy seem to speak in favour of the last-mentioned view. It is supported by ADATI (1933). NEBEL's original suggestion of the Pomoideae as halved pentaploids with the basic number 7 is, on the other hand, no longer discussed.

In his most recent work MOFFETT (1934) partially accepts SAX's views. His own description of multivalent chromosomes and presumptive conception of the apples as autopolyploids he now explains as errors in terminology; and he unreservedly accepts the allopolyploidy of the Pomoideae. Regarding the basic number of the group, his conclusion now is as follows: »The weight of evidence, therefore, points to the primary number in the Pomoideae being 7, and even if the group has arisen from a combination of the numbers 8 and 9 it is probable that these numbers themselves have been derived from the primary 7». The discussion having arrived at this point, there does no longer seem to exist any essential discrepancies in the conception of the group.

2. Evidence from Conjugation and secondary Association of the Chromosomes.

The chromosome number 17 is in itself an indication of polyploidy, as it can scarcely be a primitive basic number. Further evidence is procured by

DARLINGTON's and MOFFETT's (l. c.) discovery of four morphologically identical chromosomes in the somatic set of the »diploid» apples. The rare, though regular, occurrence of a quadrivalent chromosome at meiosis of »diploids», and the somewhat more regular occurrence of univalents, appears to be characteristic of an allopolyploid condition. Occasional multivalents and univalents may occur in allopolyploids as a result of faulty conjugation, the latter being no uncommon feature of allopolyploidy. The occurrence of quadrivalents and higher multivalents in »triploid» apples is a still further proof of the polyploidy of the so called diploids.

Secondary association has recently been shown by MEURMAN (1933) and MÜNTZING (1933) to be closely connected with chromosome homology and polyploidy. The former author found two conspicuously big gemini in *Acer platanoides,* which were always strongly associated secondarily. They obviously correspond to two identical pairs of especially long chromosomes in the somatic plates. This observation shows the secondary association to operate between (probably) homologous chromosomes. MÜNTZING studied the phenomenon in diploid, triploid and tetraploid strains of *Solanum tuberosum.* The groups of secondarily associated chromosomes were larger (comprised each more chromosomes) in the tetraploid and triploid than in the diploid: in the former generally 2—4, in the last-mentioned generally not more then 2 chromosomes each. These results indicate the original basic number in *Solanum* to be 6 and not 12, and also clearly shows the influence of polyploidy upon the secondary grouping of chromosomes at meiosis.

In view of such data the secondary association in apples and other Pomoideae must be interpreted as an outcome of chromosome homology and polyploidy (allopolyploidy), according to the theory of DARLINGTON and MOFFETT. The graphs of fig. 3 show the basic number of the group to be, most probably, 7.

In this connection reference is made to similar studies by WANSCHER (1934) on secondary association in Umbelliferae and Bicornes.

3. Evidence from Chromosome Numbers in the Progeny of Triploids.

MOFFETT (1931) reports on chromosome counts of seedlings from crosses between diploid and triploid apples. These seedlings are unusual in that the most common somatic chromosome number is 41. This is the sum of the presumed primary haploid number 7 and the secondary diploid number 34. »It is to be imputed that the high frequency of seedlings with 41 chromosomes is due to the greater viability of the 17 + 7 gametes in the triploid —», and the frequency of 41 chromosome seedlings is regarded as a proof of the primary basic number 7.

Recently NEBEL (1933) tried to test this conclusion by a statistical treatment of his own, more complete results from diploid × triploid, triploid × diploid,

and triploid × triploid crosses. He shows that the frequency polygon of chromosome numbers of the seedlings is in better harmony with expectation if 7 (and not 8 according to SAX's suggestion) is regarded as the primary set.

4. Evidence from Pollen Lethality.

The factorial interpretation of the pollen lethality in apples, given above, is altogether founded on the conception that the apples are polyploids. In the following a comparison is made with similar phenomena in some diploid plants, and a general discussion of pollen lethality presented.

There are two types of partial pollen lethality, one showing irregular proportions of abortive grains, the other showing more or less Mendelian ratios. Cases of the former type are generally caused by irregular meiosis (chromosome aberrations) and are not concerned in this connection. Cases of partial gametic lethality in Mendelian proportions have been found and analyzed in the following plants: *Stizolobium*-hybrids, *Datura stramonium* and certain *Datura*-hybrids, *Zea Mays, Pisum sativum, Campanula persicifolia,* many species and mutants of *Oenothera, Matthiola incana, Galeopsis tetrahit,* and many apple varieties (conf. HÅKANSSON 1931, SANSOME and PHILP 1932). *Matthiola* is a special case where the gametic lethal kills the pollen grains but generally not the ovules, and *Stizolobium* has not been subjected to cytological investigation. Partial gametic lethality in *Datura, Zea, Pisum, Campanula* and *Oenothera* has been found to be connected, more or less regularly, with the formation of chromosome rings at meiosis, the rings being regarded as a result of segmental interchange between non-homologous chromosomes. At meiosis of *Galeopsis* and of apple varieties, again, such rings are not found. A glance at the list of plants above immediately shows a difference between the ring-forming plants and those without rings. The former types are all diploids, the latter polyploids. The autotetraploid strains of *Datura, Campanula* and *Oenothera* may be left out of consideration, as they do not show any clear sterility relations. Thus the following conclusion may be drawn: In diploid plants gametic lethality in Mendelian proportions is connected with segmental interchange and ring-formation (except in a few special cases); in polyploid plants such a connection is generally not found. That this difference may be a mere consequence of polyploidy itself, will appear from the following discussion.

If, in a case of segmental interchange, the partners of a chromosome ring are separated according to a »non-disjunctional» scheme, all the gametes in a tetrad thus formed are deficient with regard to a piece of chromosome (two of the gametes in the tetrad with regard to one piece, two with regard to another). Consequently, all four gametes of the tetrad die. In the case of a »disjunctional» separation, again, all four gametes live (all four having a set of complete chromosomes). The same relations hold good for cases of segmental interchange without ring-formation at meiosis (PELLEW and SANSOME 1931). In all these cases a proportion of the gametes die on account of chromosome deficiencies.

That chromosome deficiencies are a common (if not universal?) cause of gametic lethality has been made probable by several recent investigations. According to PHILP and HUSKINS (1931) the pollen lethal in the ever-sporting strain of *Matthiola incana* is a deficiency visible as the lack of a satellite. The secondary trisomics of *Datura stramonium* produce a certain amount of pollen which is deficient with regard to a certain piece of chromosome and, consequently, is abortive. In cases of partial sterility in connection with segmental interchange the abortion of the gametes is clearly due to chromosome deficiencies. Numerous cases of translocations and deletions induced by X-rays should also be mentioned. For further discussion reference is made to SANSOME and PHILP (1932), SEREBROVSKY (1929) and GOODSPEED (1931). To a similar category of phenomena probably also belongs the peculiar case of *Vicia faba* where SIRKS (1931) assumes »some lethal character which is diffused through the whole chromosome». This assumption is made because no crossing-over between the lethal factor and other factors occurs. If the lethal is assumed to be a deficiency, the lack of crossing-over is, however, easily explained!

Under natural conditions chromosome deficiencies generally seem to arise from translocations through which some gametes receive a duplicated chromosome piece and others become deficient of the same piece. Translocations may be simple or reciprocal. Simple deficiencies take their origin from simple translocations (sometimes from fragmentation). A haploid gamete, produced by a diploid plant and lacking a piece of a certain chromosome (*A*), becomes abortive on account of this deficiency. Consequently, the deficiency is not transmitted to the next generation, and can thus not give rise to plants permanently segregating a certain proportion of abortive gametes. Diploid plants showing partial pollen lethality do not arise in this way. A diploid gamete, again, produced by a tetraploid plant, possesses two chromosomes *A* (it is *AA*) and a deficiency in one of these chromosomes does generally not affect the viability of the gamete (at least not much). Hence, in the course of many generations of a polyploid plant such deficiencies may accumulate as recessive gametic lethals and plants permanently segregating a regular proportion of abortive gametes may arise. Polyploid plants showing partial pollen lethality may arise in this way. Such plants will have no chromosome rings.

Reciprocal translocations, on the other hand, easily give rise to »structural hybrids» with, for instance, two chromosomes *A*—*B* and *C*—*D*; and two others *A*—*C* and *B*—*D*. Very similar would be cases of simple translocation if the duplicated and the deficient chromosome have been kept together in the same gamete and given rise to a »structural hybrid» on crossing with the normal type. If such plants are diploids, they generally segregate a certain proportion of abortive gametes, the four chromosomes representing a system of balanced lethals. Diploid plants showing partial pollen lethality arise in this way. In a polyploid, again, such a system of balanced lethals would generally not impair the viability of the gametes (or have only a slight effect), as a deficiency in one chromosome is generally counteracted by the homologous chromosome (or chro-

mosomes) present in the same gamete. An accumulation of several identical interchanges would, of course, ultimately lead to a polyploid plant with partial gametic lethality, but such an accumulation of reciprocal translocations is probably relatively rare. Polyploid plants showing partial pollen lethality do therefore not, as a rule, arise in this way.

By the above interpretation of partial pollen lethality in Mendelian proportions all cases sufficiently investigated are referred to one common cause: Translocations of pieces of chromosomes (or fragmentation). If such translocations result in systems of balanced lethals, partial pollen lethality may arise in diploids, but generally not in polyploids, if again, they result in deficiencies which accumulate as polymeric recessive lethals, partial pollen lethality may arise in polyploids, but not in diploids. This may be regarded as a general theory of partial gametic lethality in Mendelian proportions, and it makes clear the influence of polyploidy upon such lethality. Parenthetically we may add that according to BLAKESLEE and SATINA (1934) gametic lethals in plants do not excercise their influence upon the gametes themselves, but upon the gametophytes (the mature pollen grain and embryo-sac and the pollen tube). This would explain why in animals — where gametophytes are lacking — chromosome deficiencies do not act as gametic lethals (at least not in *Drosophila*). The gametic lethals in (higher) plants should thus more properly be called gametophytic lethals.

In the interchange diploids enumerated above 50 % and 75 % of pollen abortion has been met with, in *Datura* 50 % and 25 % of abortion. The last-mentioned figure is somewhat exceptional and has so far not been explained by the interchange hypothesis. Perhaps *Datura stramonium* is no real diploid but a secondarily balanced type with some of the chromosome parts duplicated. Partial pollen abortion would then, in some of the crosses, result from a cooperation of segmental interchange with a simple deficiency. This condition would also explain why in certain *Datura*-crosses a ring of four is found, but no sterility. On the whole, *Datura stramonium* might be suspected of being not quite a typical diploid. Its basic number is rather high (12), while the basic number of *Zea Mays* is 10, of *Campanula persicifolia* 8, of *Pisum sativum* and the *Oenothera*-types 7. In *Zea Mays,* too, a case of 25 % of lethality has been found. It has been clearly shown, however, that this is only a special case of translocation, connected with the formation of a chain of four, not a ring (BURNHAM 1932). The data on gametic lethality in *Campanula* are, as yet, very inadequate, and in *Oenothera* the preponderatingly »disjunctional» segregation of the large rings (zigzag arrangement) give rather irregular lethality figures. On the whole, however, the data on all these interchange diploids afford very conclusive evidence of the connection between segmental interchange, ring-formation at meiosis, and gametic lethality.

In the partially sterile line-crosses of the tetraploid *Galeopsis tetrahit* 50 % and 25 % of pollen abortion is found. There are no rings or chains at meiosis, as explicitly stated by MÜNTZING (1929, 1930, 1932). The cause of the pollen lethality is still unknown. There is no positive evidence of segmental inter-

change. An interpretation on the basis of simple translocations might be tentatively suggested. The chromosome pairs A_1A_1 and A_2A_2 may be substituted for the gene pairs AA and BB in MÜNTZING's bifactorial explanation (which is of an entirely formal nature). A_1 and A_2 are homologous chromosomes belonging to two different genoms. If, through a simple translocation, a piece has been transplanted from an A_2 to an A_1 chromosome, a duplication and a deficiency arise simultaneously. A race homozygous for both may be designated $A_1+A_1+A_2-A_2-$. All gametes produced by such a race are A_1+A_2- and they are all normally balanced and viable (as are the gametes A_1A_2 of the original race). A hybrid between the two races is $A_1A_1+A_2A_2-$. This hybrid produces four kinds of gametes: A_1A_2, A_1A_2-, A_1+A_2 and A_1+A_2-. The first and the last of these four types are balanced and viable; the two recombination gametes are unbalanced and may be abortive. If both the deficiency (A_1A_2-) and the duplication (A_1+A_2) are abortive, 50 % of pollen lethality arises, if only the deficiency is abortive, the result is 25 % of pollen lethality. All the details in MÜNTZING's purely formal explanation remain unaltered, only systems of chromosomal balance being substituted for the Mendelian systems of dominant and recessive factors. This may make the lethality of the unbalanced combinations more comprehensible and places the *Galeopsis*-case into line with the other cases of partial pollen lethality. However, the explanation is, of course, entirely hypothetical. Chromosome rings are not expected, while a chain of four might occur, if the translocated piece is not too small (which it probably is). It should be pointed out that the tetraploid constitution of *Galeopsis tetrahit* entails the presumed translocations resulting in changes of balance only, not in absolute deficiencies as in the cases of segmental interchange in diploids (this may account for a good deal of the variation found).

In the polysomic tetraploid apples 34 1/3 %, 25 % and 12 1/2 % of pollen abortion is found. The lethality figures of apples are typically polyploid and afford additional evidence of the polyploidy of the Pomoideae. The »recessive genes» *a(d)* should be regarded as chromosomes which are deficient as compared with the unaltered chromosomes *A(D)*. For the rest our factorial interpretation above (p. 165 f.) need not be altered.

5. Evidence from Incompatibility.

Apple varieties are more or less self-sterile, though complete self-sterility apparently seldom occurs. Typically self-fertile varieties do probably not occur either. Cross-incompatibility appears to be still less evident, and most varietal crosses seem to be more or less successful. There are practically continuous transitions between the various grades of compatibility and this is regarded as characteristic of polyploidy (CRANE and LAWRENCE 1929, 1931). Intra-sterile inter-fertile groups seem to be lacking. For Swedish varieties conf. CALLMAR and JOHANSSON (1934) and JOHANSSON (1934).

With regard to their practically continuous variation in compatibility, apples differ sharply not only from the diploid sweet cherries but also from the hexa-

ploid plums. Among the last-mentioned, in spite of their hexaploidy, typically self-fertile and completely self-sterile varieties are frequent, and cross-incompatibility groups may also be discerned. The very remarkable lack of distinction between the various grades of compatibility in apples may be due to a tendency of aposporic development of the seeds (GORCZYŃSKI 1934) or to the often observed tendency of parthenocarpic development of the fruit.

AFIFY (1933 *b*), in a short note, reports differential pollen tube growth in the styles of »Cox's' Orange Pippin» pollinated with pollen from »Ellison's Orange» (probably diploid). Four classes of pollen grains were observed: (a) sterile grains not growing, (b) those which put forth a short tube that soon stopped growing, (c) those which grew to one-third of the length of the style, and (d) those which reached the ovary. This behaviour is believed to be conditioned by polymeric incompatibility factors (polyploidy!). The present writer would like to point out, however, that the four classes of pollen grains might as well correspond to the four classes *aaa, Aaa, AAa* and *AAA,* according to the hypothesis on pollen lethality in this paper. What actually happens at fertilization of apples, is still unknown. An extensive study of pollen tube behaviour might prove interesting.

6. Evidence from Gene Analysis.

Recently, CRANE and LAWRENCE (1933) published a rather extensive report on genetical studies in cultivated apples. The results presented »are remarkably uniform in one particular. Almost without exception variation in the apple is practically continuous.» Clear Mendelian segregations were not met with. This behaviour is believed to be due to a strongly polymeric condition of the genes, and this, again, must be an outcome of polyploidy and of pronounced heterozygosity due to the ease with which hybridizations generally occur among Pomoideae.

E. Polyploidy and Fruit-ripening.

During recent studies on polyploidy it has often been found that autopolyploidy — generally in contradistinction to allopolyploidy — has a certain retarding effect upon growth and development. This is regarded as an unfavourable characteristic of autopolyploidy, and as autopolyploids generally also suffer from some degree of generational sterility, they have hitherto been regarded as offering a less valuable material for breeding purposes. In the following, however, some new aspects of autopolyploids are presented which should be favourable for their reputation as breeding material.

The retarding effect of autopolyploidy on the development of a plant must be supposed to extend its operation to the physiological processes in the ripening fruit. It must, consequently, be supposed to operate also during the after-ripening of fruits, and affect the possibility of storing fruit for winter use. Now, cytological research has shown the triploid apple varieties to be secondary auto-

triploids. Hence, one might suppose apples of these triploids to be, on an average, slower than the diploids in after-ripening and superior with regard to keeping quality.

In northern countries the possibility of storing fruit for winter use is a pomological problem of considerable economic importance. Swedish manuals accordingly divide our various apple varieties into three classes with regard to the above point of view: summer-apples (»sommaräpplen»), to be consumed late in the summer and in the early autumn, autumn-apples (»höstäpplen»), to be consumed in autumn, and winter-apples (»vinteräpplen») which may be kept for a greater part of the winter, sometimes even until next spring. The Swedish manuals also give data regarding the latest month until which the storage of our various kinds of apples is possible. Table VIII contains some relevant data of this kind, compiled from ENEROTH-SMIRNOFF 1896, LIND 1920—24 and PIHL and ERIKSSON (1924). Only such varieties have been included which are cultivated — or have been tested — in Sweden (so that the data may be comparable), and for which information as to diploidy *versus* triploidy is found in table I and table VI of the present work.

The table, comprising 65 apple varieties, clearly shows a strong correlation to exist between triploidy and keeping quality. About half of the numerous diploid varieties belong to the summer and autumn types and half of them to the resistant winter type, while all the twelve triploids included belong to the latter category. The conclusion is obvious: Autotriploidy has a favourable effect upon the durability and keeping quality of apples.

This remarkable physiological effect of autotriploidy — not before observed — probably implies a practical and economical problem of considerable importance. The breeding of new apple varieties, with improved keeping quality of the fruit, would be much facilitated, if the problem of producing at will new autotriploids (and autotetraploids!) could be solved in a satisfactory way.

Our knowledge of pears is too fragmentary to permit of any conclusion whatsoever regarding the present question. Besides, most pear varieties show only small differences with regard to the keeping quality of the fruit.

F. Summary of Theoretical Results.

After an introductory survey of the literature a comprehensive treatment of apple cytology is presented:

1) $^4/_5$th—$^5/_6$th of all apple varieties are diploids ($2n = 34$) and $^1/_5$th—$^1/_6$th triploids ($3n = 51$). Conf. table I.

2) In the diploids generally 17 bivalent chromosomes are formed and meiosis is quite regular. Quadrivalents occur, though they are very rare; univalents are somewhat more frequent (table II). The frequency of univalents may increase above $+30°$ C. Normally, the univalents are relatively frequent at diakinesis and appear as lagging chromosomes at anaphase I, but are almost lacking at metaphase I. It is suggested that some of the gemini at metaphase

Table VIII. **Polyploidy and After-ripening of Apples.**

2 = diploid variety. 3 = triploid variety.

Variety	*Storage possible until*									
	Sept.	Oct.	Nov.	Dec.	Jan.	Febr.	March	April	Maj	June
Astrachan, big, transp.	2									
» , Gyllenkr.	2									
» , white	2									
Belyi Naliv	2									
Calville rouge	2									
Hampus	2									
Skvoznoy Naliv	2									
Sommergewürzapfel	2									
Transp. blanche	2									
Vitgylling	2									
Aspa		2								
Astrachan, red		2								
Charlamovsky		2								
Eneroth's Klaräpple		2								
Keswick Codlin		2								
Sävstaholm		2								
Titovka		2								
Barlóvskoje			2							
Borovinka			2							
Hörningsholm			2							
Mank's Codlin			2							
Oranie			2							
Rosenhäger			2							
Stäringe			2							
Antonovka				2						
Björkvik				2						
Cox's Pomona				2						
Kavlås				2						
Alexander					2					
Cellini					2					
Frösåker					3					
Grågylling					2					
Öland's Kungsäpple					2					
Danziger Kantapfel						2				
Filippa						2				
Gravensteiner, yellow						3				
» , red						3				
Harbart's Reinette						3				

Table VIII (cont.).

Variety	*Storage possible until*									
	Sept.	Oct.	Nov.	Dec.	Jan.	Febr.	March	April	May	June
Maglemer						2				
Muskat-Reinette						2				
Baumann's Reinette							2			
Belleflower, yellow							2			
Blenheim Orange							3			
Husmodersäpple							3			
Melon							2			
Signe Tillisch							2			
Wealthy							2			
Winter Golden Pearmain							2			
Winter-Zitronenapfel							3			
Yellow Richard							2			
Cox's Orange Pippin								2		
Rossvik								3		
Stenkyrka								2		
Svanetorp								2		
Åkerö									2	
Blanche d'Espagne									2	
Canadian Reinette									3	
Pearmain d'Adam									2	
Reinette de Champagne									2	
» d'Orléans									2	
Rhode Island Greening									3	
Kasseler Reinette										2
Ribston										3
Roter Eiserapfel										3
» Stettiner										2

	Sept.		Oct.		Nov.		Dec.		Jan.		Febr.		March		April		May		June	
	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n
Total	10	—	7	—	7	—	4	—	4	1	4	3	7	3	3	1	5	2	2	2
% triploids	—		—		—		—		20		43		30		25		29		50	
Grand total	28 diploids								25 diploids and 12 triploids											

I are formed without chiasma-formation (»pseudo-gemini»). The chromosome conjugation of the diploid apples is regarded as indicating allopolyploidy.

3) The literature on secondary chromosome association is fairly fully discussed, and new data on apples are provided (table III). DARLINGTON's and MOFFETT's results on secondary association are wholly confirmed. The chromosome formula of the apple is *AAABBBCCCDDEEFFGG*.

4) The literature on meiosis in triploid apples is reviewed and some additional data are furnished (table IV). It is stated that the triploid apples are (secondary) autotriploids.

An extensive treatment is published on pollen lethality in diploid apples. The following are the main results:

1) Diploid apples may be divided into fairly distinct classes with regard to pollen abortion. Class *A*: Fully fertile pollen. Class *B*: 7/8th of the pollen viable and 1/8th abortive (empty grains). Class *C*: 3/4th viable and 1/4th abortive grains. Class *D*: Probably a combination of *B* and *C* with 7/8 . 3/4 or about 65 % of viable pollen. Conf. table VI and the graphs of figs. 6 and 7.

2) High temperatures have a very slight influence on meiosis below + 30° C., but act injuriously upon the post-meiotic maturing of the pollen (graphs figs. 8 and 9). Varieties of class *B* appear to be, throughout, very sensitive at this period, those of class *A* and »Cellini» (class *C*) are slightly sensitive or practically insensitive. A good deal of the variation in pollen viability of diploid apples is obviously due to the influence of high temperature during the post-meiotic period.

3) A factorial explanation is offered, according to which pollen abortion in diploid apples is due to systems of recessive pollen lethals. The same lethals are probably also, in semi-lethal combinations, responsible for the sensitiveness to high temperatures. The factorial explanation is based upon the formula of polyploidy presented by DARLINGTON and MOFFETT. The pollen lethals may be regarded as simple chromosome deficiencies.

4) Some data are added on pollen abortion in triploid apples.

A general survey is presented regarding the theory of secondary polyploidy in apples, as well as a discussion of the influence of polyploidy upon partial pollen lethality.

The influence of polyploidy upon after-ripening and keeping quality of apples is discussed. It is found that of the 65 varieties, contained in table VIII, 28 diploids belong to the early and less durable summer- and autumn-apples, while 25 diploids and all the 12 triploids of the table are of the late and durable winter-apple type (and late autumn type). Thus triploidy (autotriploidy!) has a favourable influence upon the keeping quality of the apples and the possibility of their storage for winter use.

G. **Some Practical Conclusions.**

From a practical point of view the triploids should be regarded separately. They have a bad pollen with a very decreased power of germination. However, according to CRANE and LAWRENCE (1931) this is to some extent counteracted

by the »greater variety in the gametic output of triploid than of diploid varieties, thereby providing a greater chance of compatible combinations». In other words, incompatibility ought to be less pronounced among triploids than among diploids. As pollen producers triploids are, however, of inferior value.

As to the pollen production of the diploids, varieties of class *A* produce the greatest quantities of viable pollen. Of importance in this connection is not only the percentage of good pollen, but also the absolute quantity produced. In a recent paper the writer (HEILBORN 1933 *a*) showed that »P. J. Bergius» produces about 250,000 viable pollen grains in each flower, »Cellini» about 150,000, and »Åkerö» about 90,000 viable grains in each flower. »Cellini» is thus a good pollen producer in spite of its rather low viability percentage. Another circumstance to be taken into consideration is the different sensitiveness to high temperatures. In this respect the *B*-varieties appear to be less suitable as pollen producers. It is true that the pollen quantities produced by the *B*-varieties may be sufficient in spite of the abortion of a considerable proportion of the grains, but this is scarcely proved by the pollination experiments that have been carried out. When flowers are pollinated experimentally, such a vast excess of pollen is used as to wipe out completely all minor differences in pollen production, but these minor differences may, nevertheless, be of importance under the natural conditions of insect pollination. If the abortion of 20—30 % of the pollen might reduce the fruit crop with, for instance, 5—7 %, the pollen abortion may be of economic importance. For a better understanding of the pollen production of the *B*-varieties comparative studies on pollen tube growth in the styles should be made with these varieties and with the *A*-varieties.

According to RIABOV (1930) »excessively high temperature (over 24°—25° C.) during flowering is more fatal for the pollen than a low temperature which sometimes only retards the germination of the pollen without depriving it if its vitality». »Cold weather during flowering, on the other hand, may prevent fertilization by retarding the growth of the pollen tubes.»

Provisionally the following advice may be given:

1) Diploids and triploids should be planted together in order to make use of both the good pollen production of the former and the favourable compatibility relations of the latter.

2) As pollen producers the *A*-varieties should be used in the first place.

3) Table I gives information as to triploids and the graph of fig. 7 as to *A*-varieties.

4) With regard to breeding of new apple varieties with improved keeping quality of the fruit, triploids deserve special attention.

Stockholm, Bot. Inst. of the University, April 1935.

Bibliography.

ADATI, S., 1933. Untersuchungen über die Chromosomenzahlen bei asiatischen wilden *Pyrus*-Arten. — Cytologia, Bd. 4.

AFIFY, A., 1933 *a*. The cytology of the hybrid between *Lycopersicum esculentum* and *L. racemigerum* in relation to its parents. — Genetica, XV.

——, 1933 *b*. Pollen tube growth in diploid and polyploid fruits. — Journ. of Pomol. and Hort. Science, Vol. XI.

BERGMAN, B., 1935. Zytologische Studien über die Fortpflanzung bei den Gattungen *Leontodon* und *Picris*. — Svensk Bot. Tidskr., Bd. 29.

BLAKESLEE, A. F., and SATINA, S., 1934. Les plantes diffèrent-elles des animaux par des gamètes léthals? — Comptes rend. des séances de l'Acad. des Sciences, T. 198.

BURNHAM, C. R., 1932. An interchange in maize giving low sterility and chain configurations. — Proc. Nat. Acad. of Sciences, Vol. 18.

CALLMAR, G., och JOHANSSON, E., 1934. Pollinering och fruktsättning hos äpplesorter. — Sveriges Pom. För:s Årsskrift, Årg. 35.

CATCHESIDE, D. G., 1932. The chromosomes of a new haploid *Oenothera*. — Cytologia, Vol. 4.

——, 1934. The chromosomal relationships in the swede and turnip groups of *Brassica*. — Ann. of Bot., Vol. XLVIII.

CRANE, M. B., and LAWRENCÉ, W. J. C., 1929. Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. — Journ. of Pomol. and Hort. Science, Vol. VII.

——, 1930 *a*. Fertility and vigour of apples in relation to chromosome numbers. — Journ. of Gen., Vol. XXII.

——, 1930 *b*. Studies in sterility. — IXth Int. Hort. Congress. London.

——, 1931. Sterility and incompatibility in diploid and polyploid fruits. — Journ. of. Gen., Vol. XXIV.

——, 1933. Genetical studies in cultivated apples. — Journ. of Gen., Vol. XXVIII.

DARLINGTON, C. D., and MOFFETT, A. A., 1930. Primary and secondary chromosome balance in *Pyrus*. — Journ. of Gen., XXII.

ELSSMANN, E., und v. VEH, R., 1931. Beiträge zur Frage nach den Befruchtungsverhältnissen der für Deutschland wirtschaftlich wertvollsten Kern-, Stein- und Beerenobstsorten. I. Nachweis der Reduktionsteilung im weiblichen Archespor von *Malus* (bei der Sorte »Schöner von Boskoop»). — Die Gartenbauwissenschaft, Bd. 6.

ENEROTH, O., 1896. Handbok i svensk pomologi. Ny uppl. af A. SMIRNOFF. Andra delen. Äpplen. — Stockholm (Norstedt).

FLORIN, R., 1927. Pollen production and incompatibilities in apples and pears. — Mem. Hort. Soc. of New York, 3.

GOODSPEED, T. H., 1931. Die Bedeutung von quantitativen Chromosomenveränderungen. — Die Naturwissenschaften, Jahrg. 19.

GORCZYŃSKI, T., 1934. Untersuchungen über die Entwicklung der Samenanlage und des Embryosackes bei der Apfelsorte »Schöner von Boskoop». — Acta Soc. Bot. Pol., Vol. XI, Suppl.

GUSTAFSSON, Å., 1934. Secondary association between homologous univalents. — Svensk Bot. Tidskr., Bd. 28.

——, 1935. Primary and secondary association in *Taraxacum* — Hereditas, XX.

HÅKANSSON, A., 1931. Über Chromosomenverkettung in *Pisum*. — Hereditas, XV.

HEILBORN, O., 1928. Zytologische Studien über Pollensterilität von Apfelsorten. — Svensk Bot. Tidskr., Bd. 22.

——, 1930. Temperatur und Chromosomenkonjugation. — Svensk Bot. Tidskr., Bd. 24.

HEILBORN, O., 1932. Lethal gene-combinations and pollen sterility in diploid apple varieties. A critique and a theory. — Hereditas, XVI.

——, 1933 *a*. Über Eutelie oder Zellkonstanz in pflanzlichen Geweben (Methoden und Probleme). — Svensk Bot. Tidskr., Bd. 27.

——, 1933 *b*. Om olika slag av sterilitet hos fruktträden. — Allm. Svensk Trädgårdstidn., Årg. 1933.

HELM, URSULA, 1934. Über die Gesetzmässige Lage der Gemini im Kernraum. — Zeitschr. für Zellforsch. und mikroskop. Anat., Bd. 21.

HOLLINGSHEAD, LILLIAN, 1932. The occurrence of unpaired chromosomes in hybrids between varieties of *Triticum vulgare*. — Cytologia, Vol. 3.

HOWLETT, F. S., 1932. The relationship of chromosomal irregularities in megasporogenesis to the fertility and fruitfulness of varieties of *Malus*. — Proc. 6th Int. Congr. Genet., Ithaca, N. Y., Vol. 2.

JOHANSSON, E., 1934. Pollinering och fruktsättning hos äpplesorter. — Sveriges Pom. För:s Årsskrift, Årg. 35.

KOBEL, F., 1926. Die zytologischen Ursachen der partiellen Pollensterilität bei Apfel- und Birnsorten. — Archiv der Julius Klaus-Stift., Bd. II.

——, 1927. Zytologische Untersuchungen an Prunoideen und Pomoideen. — Archiv der Julius Klaus-Stift., Bd. III.

——, 1931. Lehrbuch des Obstbaus auf physiologischer Grundlage. — Berlin (Springer).

LAWRENCE, W. J. C., 1930. Incompatibility in polyploids. — Genetica, XII.

——, 1931. The secondary association of chromosomes. — Cytologia, Vol. 2.

LILJEFORS, A., 1934. Über normale und apospore Embryosackentwicklung in der Gattung *Sorbus*, nebst einigen Bemerkungen über die Chromosomenzahlen. — Svensk Bot. Tidskr., Bd. 28

LIND, G., 1920—24. Svensk frukt. — Stockholm (Bergvall).

LINDSTROM, E. W., and HUMPHREY, L. M., 1933. Comparative cyto-genetic studies of tetraploid tomatoes from different origins. — Genetica, 18.

MEURMAN, O., 1933. Chromosome morphology, somatic doubling and secondary association in *Acer platanoides* L. — Hereditas, XVIII.

MIEDZYRZECKI, CH., 1933, Études cytologiques et stérilité du pollen chez le Pommier et le Poirier. — Comptes rend. des séances de la Soc. de Biol., Tome CXIV.

——, 1934. Stérilité du pollen chez les arbres fruitiers. — Congr. Int. d'Arboriculture fruit. et de Pomol., Casablanca 1934.

MOFFETT, A. A., 1931. The chromosome constitution of the Pomoideae. — Proc. Royal Soc., B, Vol. 108.

——, 1934. Cytological studies in cultivated pears. — Genetica, XV.

MÜNTZING, A., 1929. Cases of partial sterility in crosses within a Linnean species. — Hereditas, XII.

——, 1930. Outlines to a genetic monograph of the genus *Galeopsis*. — Hereditas, XIII.

——, 1932. Disturbed segregation ratios in *Galeopsis* caused by intraspecifiic sterility. — Hereditas, XVI.

——, 1933. Studies on meiosis in diploid and triploid *Solanum tuberosum* L. — Hereditas, XVII.

NATIVIDADE, J. V., 1932. A improdutividade em pomologia. — Lisboa. (Not seen!)

NEBEL, B. R., 1929 *a*. Zur Zytologie von *Malus* und *Vitis*. — Die Gartenbauwissenschaft, Bd. 1.

——, 1929 *b*. Chromosome counts in *Vitis* and *Pyrus*. — Amer. Nat., Vol. 63.

——, 1929 *c*. Zur Zytologie von *Malus*. II. — Der Züchter, Bd. 1.

——, 1930. Recent findings in cytology of fruits (Cytology of *Pyrus* III). — Proc. Amer. Soc. for Hort. Science, 1930.

NEBEL, B. R., 1933. Chromosome numbers in aneuploid apple seedlings. — New York State Agric. Exp. Station, Geneva, N. Y., Techn. Bull., No. 209.

PELLEW, C., and SANSOME, E. R., 1931. Genetical and cytological studies on the relations between Asiatic and European varieties of *Pisum sativum*. — Journ. of Gen., Vol. XXV.

PHILP, J., and HUSKINS, C. L., 1931. The cytology of *Matthiola incana* R. Br., especially in relation to the inheritance of double flowers. — Journ. of Gen., Vol. XXIV.

PIHL, A., och ERIKSSON, J., 1924. Svenska fruktsorter. 2:a uppl. — Stockholm (Norstedt).

RIABOV, J. V., 1930. Pollination and fruiting of fruit trees. I. Survey of the literature. — Journ. Gov. Bot. Gard., Nikita, Yalta, Vol. XIV.

ROSCOE, MURIEL, 1933. The chromosomal constitution of certain cultivated apple varieties. — Journ. of Gen., Vol. XXVIII.

RYBIN, V. A., 1926. Cytological investigations of the genus *Malus* (preliminary account). — Bull. of Appl. Bot. and Plant Breeding, Vol. 16.

——, 1927. On the number of chromosomes observed in the somatic and reduction division of the cultivated apple in connection with pollen sterility of some of its varieties. — Bull. of Appl. Bot. and Plant Breeding, Vol. 17.

SANSOME, F. W., and PHILP, J., 1932. Recent advances in plant genetics. — London (Churchill).

SAX, K., 1931. The origin and relationships of the Pomoideae. — Journ. Arnold Arbor., Vol. XII.

——, 1932. Chromosome relationships in the Pomoideae. — Journ. Arnold Arbor., Vol. XIII.

——, 1933. The origin of the Pomoideae. — Amer. Journ. Hort. Sci., 30. (Not seen!)

SEREBROVSKY, A. S., 1929. A general scheme for the origin of mutations. — Amer. Nat., Vol. 63.

SHOEMAKER, J. S., 1926. Pollen development in the apple, with special reference to chromosome behaviour. — Bot. Gaz., Vol. LXXXI.

SIRKS, M. J., 1931. Plasmatic influences upon the inheritance in *Vicia Faba*. I. The elimination of a whole linkage group in the plasm of *Vicia Faba minor*. — Proc. Kon. Akad. van Wetensch. te Amsterdam, Vol. XXXIV.

STEINEGGER, P., 1932. Zytologisch bedingte Ei- und Zygotensterilität bei triploiden Apfelsorten. — Ber. Schweiz. Bot. Ges., Bd. 41.

v. VEH, R., 1933 *a*. Beiträge zur Frage nach den Befruchtungsverhältnissen der für Deutschland wertvollsten Kern-, Stein- und Beerenobstsorten. II. Entwicklungsgeschichtlich-cytologische Untersuchung der Samenanlagen der Apfelsorte »Schöner von Boskoop». — Die Gartenbauwissenschaft, Bd. 8.

——, 1933 *b*. Ergebnisse einer entwicklungsgeschichtlich-cytologischen Untersuchung der Samenanlagen der Apfelsorte »Schöner von Boskoop». — Der Züchter, Jahrg. 5.

WANSCHER, J. H., 1934. Secondary (chromosome) associations in Umbelliferae and Bicornes. — The new Phytologist, Vol. XXXIII.

WILSON, E. B., 1925. The cell in development and heredity. Third ed. — New York (Macmillan).

ZIEGLER, A., und BRANSCHEIDT, P., 1927. Pollenphysiologische Untersuchungen an Kern- und Steinobstsorten in Bayern und ihre Bedeutung für den Obstbau. — Berlin (Parey).

Printed $^{18}/_{7}$ *1935.*